10/573998

BCS 03-5004-PCT

IAP20 Rec'd PCT/PTO 3 0 MAR 2006

BCS 03-5004

Bayer CropScience GmbH

Plants with reduced activity of a Class 3 branching enzyme

Description

The present invention relates to plant cells and plants, which are genetically modified, wherein the genetic modification leads to the reduction of the activity of a Class 3 vegetable branching enzyme in comparison with corresponding wild type plant cells or wild type plants that have not been genetically modified. Furthermore, the present invention relates to means and methods for the manufacture of such plant cells and plants. Plant cells and plants of this type synthesise a modified starch. The present invention therefore also relates to the starch synthesised by the plant cells and plants according to the invention as well as to methods for the manufacture of the starch and to the manufacture of starch derivatives of this starch. Furthermore, the present invention relates to nucleic acids coding a Class 3 branching enzyme, vectors, host cells, plant cells and plants containing such nucleic acid molecules.

With regard to the increasing importance currently attributed to vegetable constituents as renewable raw material sources, one of the tasks of biotechnological research is to endeavour to adapt these vegetable raw materials to suit the requirements of the processing industry. Furthermore, in order to enable regenerating raw materials to be used in as many areas of application as possible, it is necessary to achieve a large variety of materials.

Polysaccharide starch is made up of chemically uniform base components, the glucose molecules, but constitutes a complex mixture of different molecule forms, which exhibit differences with regard to the degree of polymerisation and branching, and therefore differ strongly from one another in their physical-chemical characteristics. Discrimination is made between amylose starch, an essentially unbranched polymer made from α-1,4-glycosidically linked glucose units, and the

amylopectin starch, a branched polymer, in which the branches come about by the occurrence of additional α-1,6-glycosidic links. A further essential difference between amylose and amylopectin lies in the molecular weight. While amylose, depending on the origin of the starch, has a molecular weight of $5 \times 10^5 - 10^6$ Da, that of the

5   amylopectin lies between 10^7 and 10^8 Da. The two macromolecules can be differentiated by their molecular weight and their different physical-chemical characteristics, which can most easily be made visible by their different iodine bonding characteristics.

10   Amylose has long been looked upon as a linear polymer, consisting of α-1,4-glycosidically linked α-D-glucose monomers. In more recent studies, however, the presence of α-1,6-glycosidic branching points (ca. 0.1%) has been shown (Hizukuri and Takagi, Carbohydr. Res. 134, (1984), 1-10; Takeda et al., Carbohydr. Res. 132, (1984), 83-92).

15

Amylopectin constitutes a complex mixture of differently branched glucose chains. In contrast to amylose, amylopectin is more strongly branched. According to textbook information (Voet and Voet, Biochemistry, John Wiley & Sons, 1990), on average, the α-1,6 branches occur every 24 to 30 glucose residues. This is equivalent to a degree

20   of branching of ca. 3% - 4%. The figures for the degree of branching are variable and are dependent on the origin (e.g. plant species, plant type etc.) of the appropriate starch. In typical plants used for the industrial production of starch, such as maize, wheat or potato, for example, the synthesised starch consists of ca. 20% - 30% amylose starch and ca. 70% - 80% amylopectin starch.

25

The functional characteristics of the starch, along with the amylose/amylopectin ratio and the phosphate content, are strongly affected by the molecular weight, the pattern of the side chain distribution, the ion concentration, the lipid and protein content, the average grain size of the starch and the grain morphology of the starch etc. At the

30   same time, by way of example, the solubility, the retrogradation behaviour, the water bonding capability, the film formation characteristics, the viscosity, the sticking characteristics, the freezing-thawing stability, the acid stability, the gelling strength

etc. must be mentioned as important functional characteristics. The grain size of the starch can also be important for different applications.

Branching enzymes, which are also abbreviated by the designation "BE" (from
5   Branching Enzyme; E.C. 2.4.1.18), catalyse the introduction of α-1,6 branches in α-1,4-glucans. Branching enzymes and the nucleic or amino acid sequences that characterise them are known from widely different organisms, such as bacteria, microbial fungi, mammals, algae and higher plants, for example. As only plants synthesise starch, while the above-mentioned non-vegetable organisms (e.g.
10 bacteria, fungi and mammals) synthesise glycogen, the related branching enzymes, which are involved in the synthesis of the appropriate polymer, can also be subdivided into glycogen branching enzymes and starch branching enzymes. Plants are therefore starch branching enzymes, which are often also referred to as Q-enzymes in older literature.

15

In all plant species that have been investigated up to now, the branching enzymes described can be associated with two different classes (Burton et al., 1995, Plant Journal 7, 3-15; Mizuno et al., 2001, Plant Cell Physiol. 42(4), 349-357). The association with these classes, sometimes designated in the literature with A or 2
20 respectively and B or 1 respectively, is based on the comparison of derived protein sequences.

As different nomenclatures have been used in the past for designating and classifying branching enzymes, Smith-White and Preiss (1994, Plant Molecular
25 Biology Reporter 12, 67-71) (1994, Plant Molecular Biology Reporter 12, 67-71) have proposed a system for standardising this nomenclature, in which the association with the two classes of vegetable branching enzymes is also based on the comparison of derived protein sequences (Larsson et al., 1998, Plant Mol. Biol. 37, 505-511). According to this nomenclature, those vegetable branching enzymes, the amino acid
30 sequence of which has a higher degree of identity with that of branching enzyme I of maize (GenBank Acc: D11081), is to be designated as a Class 1 branching enzyme, and those vegetable branching enzymes, the coding amino acid sequence of which has a higher degree of identity with that of branching enzyme II of maize (GenBank

Acc: AF072725), "is to be designated as a Class 2 branching enzyme. The designation of gene products, which are coding for branching enzymes, are, in accordance with the nomenclature of Smith-White and Preiss, to be incorporated in the already existing nomenclature by means of E.C. numbers. This results in the so-

5   called GPN (Gene Product Number) Codes for the two classes, namely GPN 2.2.4.1.18:1 for Class 1 branching enzymes and GPN 2.2.4.18:2 for Class 2 branching enzymes.

The following vegetable or starch branching enzymes therefore belong to Class 1

10   (GPN 2.2.1.18:1) according to the nomenclature proposed by Smith-White and Preiss (1994, Plant Molecular Biology Reporter 12, 67-71):
BE I from *Aegilops tauschii* (GenBank Acc: AF525746), BE I from barley (GenBank Acc: AY304541), BE from tapioca (GenBank Acc: X77012), BE I (frequently also described as BE 1) from rice (GenBank Acc: D11082, D10752, D10838), BE 3 from

15   bean (GenBank Acc: AB029549), BE II from pea (GenBank Acc: X80010), BE from millet (GenBank Acc: AF169833), BE I from potato (GenBank Acc: Y08786, X69805), BE from wheat (GenBank Acc: Y12320, AF076679, AF002820) and BE I from maize (GenBank Acc: D11081, AAO20100, E03435, AY176762, U17897, AF072724).
At the same time, the amino acid sequences for different Class 1 branching enzymes

20   each have an identity of more than 60% with the amino acid sequence of branching enzyme I from maize (GenBank Acc: D11081).

Branching enzymes, which belong to Class 2 (GPN 2.2.1.18:2) according to the nomenclature proposed by Smith-White and Preiss (1994, Plant Molecular Biology

25   Reporter 12, 67-71) are, for example, BE IIa from *Aegilops tauschii* (GenBank Acc: AF338431, WO 9914314), BE2-1 and BE2-2 from *Arabidosis thaliana* (BE2-1 GenBank Acc: NM_129196 CAA04134; BE2-2 GenBank Acc: CAB82930, NM_120446), BE IIa and BE IIb from barley (BE IIa GenBank Acc: AF064560; BE IIb GenBank Acc: AF064561), BE II from sweet potato (GenBank Acc: AB071286), BE

30   III and BE IV (frequently also described as BE 3 or BE 4 respectively) from rice (BE III GenBank Acc: D16201; BE IV GenBank Acc: AB023498), BE 1 from bean (GenBank Acc: AB029548), BE I from pea (GenBank Acc: X80009), BE IIb from millet (GenBank Acc: AY304540), BE II from potato (GenBank Acc: AJ000004,

AJ011885, AJ011888, AJ011889, AJ011890), BE II or BE IIa from wheat (GenBank Acc: Y11282, AF286319, AF338432, U66376) and BE II, or BE IIb from maize (BE II GenBank Acc: AAA18571, T02981; BE IIb GenBank Acc: AF072725, L08065). At the same time, the amino acid sequences for different Class 2 branching enzymes each

5 have an identity of more than 60% with the amino acid sequence of branching enzyme IIb from maize (GenBank Acc: AF072725).

Vegetable or starch branching enzymes belong to the family of alpha-amylolytic enzymes (Svensson, 1994, Plant Molecular Biology 25, 141-157; Jespersen et al.,

10 1991, Biochem J. 280, 51-55) and, with regard to the amino acid sequence, have four conserved domains (Baba et al., 1991, Biochem. Biophys. Res. Commun. 181(1), 87-94; Kuriki et al., 1996, J. of Protein Chemistry 15(3), 305-313).

Structural predictions based on mathematical calculations derived from experimental

15 data such as protein crystal structures (Pfam: http://hits.isb-sib.ch/cgi-bin/PFSCAN?) show that all previously known branching enzymes from higher plants have two domains: an alpha-amylase domain and an iso-amylase domain. Here, the iso-amylase domain lies closer to the N-terminus of the protein than the alpha-amylase domain.

20

Plants are known, for example, which have a reduced activity of a Class 2 branching enzyme due to a mutation. These include the so-called "amylose extender" (ae) mutants from maize (Stindard et al., 1993, Plant Cell 5, 1555-1566; Boyer and Preiss, 1978, Biochem. Biophys. Res. Commun. 80, 169-175) and rice (Mizuno et al.,

25 1993, J. Biol. Chem. 268, 19084-19091), as well as the "rugosus" (r) mutation in pea (Smith, 1988, Planta 175, 270-279; Bhattacharyya et al., 1990, Cell 60, 115-122). All these mutants are distinguished by the fact that they synthesise a starch, which has an increased amylose content in comparison with starches from corresponding plants, which do not have this mutation.

30

Furthermore, genetically modified potato plants are described, in which the activity of a BE I (Class 1) branching enzyme (Kossmann et al., 1991, Mol Gen Genet 230, 39-44; Safford et al., 1998, Carbohydrate Polymers 35, 155-168), or the activity of a BEII

(Class 2) branching enzyme (Jobling et al., 1999, The Plant Journal 18), or the activity of a BEI and BEII branching enzyme (Schwall et al., 2000, Nature Biotechnology 18, 551- 554, Jobling et al., 2003, Nature Biotechnology 21, 77-80) are reduced.

5

Previously, it has been possible to associate all vegetable branching enzymes to one or both of the classes described above. Plant cells or plants, which have a reduced activity of a branching enzyme, which cannot be associated with these classes, are unknown.

10

The object of the present invention is therefore based on providing modified starches, new plant cells and/or plants, which synthesise such a modified starch, as well as means and methods for producing said plants.

15   This problem is solved by the embodiments described in the claims.

The present invention therefore relates to genetically modified plant cells and genetically modified plants, characterised in that the plant cells or plants have a reduced activity of at least one Class 3 branching enzyme in comparison with 20   corresponding wild type plant cells or wild type plants that have not been genetically modified.

A first aspect of the present invention relates to a plant cell or plant, which is genetically modified, wherein the genetic modification leads to the reduction of the 25   activity of at least one Class 3 branching enzyme in comparison with corresponding wild type plant cells or wild type plants that have not been genetically modified.

At the same time, the genetic modification can be any genetic modification, which leads to a reduction of the activity of at least one Class 3 branching enzyme in 30   comparison with corresponding wild type plant cells or wild type plants that have not been genetically modified.

In conjunction with the present invention, the term "wild type plant cell" means that the plant cells concerned were used as starting material for the manufacture of the plant cells according to the invention, i.e. their genetic information, apart from the introduced genetic modification, corresponds to that of a plant cell according to the

5   invention.

In conjunction with the present invention, the term "wild type plant" means that the plants concerned were used as starting material for the manufacture of the plants according to the invention, i.e. their genetic information, apart from the introduced

10   genetic modification, corresponds to that of a plant according to the invention.

In conjunction with the present invention, the term "corresponding" means that, in the comparison of several objects, the objects concerned that are compared with one another have been kept under the same conditions. In conjunction with the present

15   invention, the term "corresponding" in conjunction with wild type plant cell or wild type plant means that the plant cells or plants, which are compared with one another, have been raised under the same cultivation conditions and that they have the same (cultivation) age.

20   In an embodiment of the present invention, the genetic modification of the plant cells according to the invention or the plants according to the invention is brought about by mutagenesis of one or more genes. The type of mutation is not important, as long as it leads to a reduction in the activity of a Class 3 branching enzyme.

25   In conjunction with the present invention, the term "mutagenesis" is to be understood to mean any type of introduced mutation, such as deletions, point mutations (nucleotide exchanges), insertions, inversions, gene conversions or chromosome translocations, for example.

30   Here, the mutation, which leads to the reduction of the activity of at least one endogenous Class 3 branching enzyme, can be produced by the use of chemical agencies or energy-rich radiation (e.g. x-rays, neutron radiation, gamma radiation or UV radiation).

Agencies, which can be used to produce chemically induced mutations, and the mutations resulting from the effect of the corresponding mutagens are, for example described in Ehrenberg and Husain, 1981, (Mutation Research 86, 1-113), Müller, 1972 (Biologisches Zentralblatt 91 (1), 31-48). The production of rice mutants using

5   gamma radiation, ethyl methane sulphonate (EMS), N-methyl-N-nitrosurea or sodium azide (NaN_3) is described, for example, in Jauhar and Siddiq (1999, Indian Journal of Genetics, 59 (1), 23-28), in Rao (1977, Cytologica 42, 443-450), Gupta and Sharma (1990, Oryza 27, 217-219) and Satoh and Omura (1981, Japanese Journal of Breeding 31 (3), 316-326). The production of wheat mutants using NaN_3 or maleic

10   hydrazide is described in Arora et al. (1992, Annals of Biology 8 (1), 65-69). An overview of the production of wheat mutants using different types of energy-rich radiation and chemical agencies is presented in Scarascia-Mugnozza et al. (1993, Mutation Breeding Review 10, 1-28). Svec et al. (1998, Cereal Research Communications 26 (4), 391-396) describes the use of N-ethyl-N-nitrosurea for

15   producing mutants in triticals. The use of MMS (methyl methane sulphonic acid) and gamma radiation for the production of millet mutants is described in Shashidhara et al. (1990, Journal of Maharashtra Agricultural Universities 15 (1), 20-23).

The manufacture of mutants in plant species, which mainly propagate vegetatively,

20   has been described, for example, for potatoes, which produce a modified starch (Hovenkamp-Hermelink et al. (1987, Theoretical and Applied Genetics 75, 217-221) and for mint with increased oil yield or modified oil quality (Dwivedi et al., 2000, Journal of Medicinal and Aromatic Plant Sciences 22, 460-463).

All these methods are basically suitable for manufacturing the plant cells according to

25   the invention and the plants according to the invention.

Mutations in the appropriate genes, in particular in genes coding a Class 3 branching enzyme, can be found with the help of methods known to the person skilled in the art. In particular, analyses based on hybridisations with probes (Southern Blot),

30   amplification by means of polymerase chain reaction (PCR), sequencing of related genomic sequences and the search for individual nucleotide exchanges can be used for this purpose. A method of identifying mutations based on hybridisation patterns is, for example, the search for restriction fragment length differences (Restriction

Fragment Length Polymorphism, RFLP) (Nam et al., 1989, The Plant Cell 1, 699-705; Leister and Dean, 1993, The Plant Journal 4 (4), 745-750). A method based on PCR is, for example, the analysis of amplified fragment length differences (Amplified Fragment Length Polymorphism, AFLP) (Castiglioni et al., 1998, Genetics 149, 2039-

5   2056; Meksem et al., 2001, Molecular Genetics and Genomics 265, 207-214; Meyer et al., 1998, Molecular and General Genetics 259, 150-160). The use of amplified fragments cut with restriction endonucleases (Cleaved Amplified Polymorphic Sequences, CAPS) can be called upon for the identification of mutations (Konieczny and Ausubel, 1993, The Plant Journal 4, 403-410; Jarvis et al., 1994, Plant Molecular

10   Biology 24, 685-687; Bachem et al., 1996, The Plant Journal 9 (5), 745-753). Methods for the determination of SNPs have been described by Qi et al. (2001, Nucleic Acids Research 29 (22), e116) Drenkard et al. (2000, Plant Physiology 124, 1483-1492) and Cho et al. (1999, Nature Genetics 23, 203-207) amongst others. Methods, which allow several plants to be investigated for mutations in certain genes

15   in a short time, are particularly suitable. Such a method, so-called TILLING (Targeting Induced Local Lesions IN Genomes), has been described by McCallum et al. (2000, Plant Physiology 123, 439-442).

These methods are basically suitable for identifying plant cells according to the
20   invention and plants according to the invention.

Hoogkamp et al. (2000, Potato Research 43, 179-189) have manufactured stable monoploid mutants starting from a potato mutant (amf), which was manufactured by means of chemical mutagens. These plants do not synthesise any more active

25   enzyme for a starch synthesis connected to the starch grain (GBSS I) and therefore produce an amylose-free starch. The monoploid potato plants obtained can be used as starting material for further mutageneses in order to identify plants, which synthesise a starch with modified characteristics. The plant cells according to the invention and plants according to the invention, which produce a starch according to

30   the invention, can be identified and isolated by appropriate methods.

The plant cells according to the invention and the plants according to the invention have a reduction of the activity of at least one Class 3 branching enzyme in

comparison with corresponding wild type plant cells that have not been genetically modified.

Here, within the framework of the present invention, the term "reduction of activity" means a reduction in the expression of endogenous genes, which code Class 3 branching enzymes, and/or a reduction in the quantity of protein of a Class 3 branching enzyme in the plant cells and/or a reduction in the enzymatic activity of Class 3 branching enzymes in the plant cells.

The reduction in the expression can, for example, be determined by measuring the quantity of transcripts coding Class 3 branching enzyme, e.g. using Northern blot analysis or RT-PCR. Here, a reduction preferably means a reduction in the amount of transcripts in comparison with corresponding plant cells that have not been genetically modified by at least 50%, in particular by at least 70%, preferably by at least 85% and particularly preferably by at least 95%.

The reduction in the amount of protein of a Class 3 branching enzyme, which results in a reduced activity of this protein in the plant cells concerned, can, for example, be determined by immunological methods such as Western blot analysis, ELISA (Enzyme Linked Immuno Sorbent Assay) or RIA (Radio Immune Assay). Here, a reduction preferably means a reduction in the amount of Class 3 branching enzyme protein in comparison with corresponding plant cells that have not been genetically modified by at least 50%, in particular by at least 70%, preferably by at least 85% and particularly preferably by at least 95%.

Within the framework of the present invention, the term "branching enzyme" (α-1,4-glucan: α-1,4- glucan 6-glycosyltransferase, E.C. 2.4.1.18) is understood to mean a protein, which catalyses a transglycosylation reaction, in which α-1,4 links of an α-1,4-glucan donor are hydrolysed and the thereby released α-1,4-glucan chains are transferred to an α-1,4-glucan acceptor chain and, in doing so, are transformed into α-1,6-links. In particular, within the framework of the present invention, the term

"branching enzyme" is to be understood to mean a vegetable branching enzyme, i.e. a starch branching enzyme.

The activity of a branching enzyme can be demonstrated, for example, with the help
5   of native acrylamide gel electrophoresis. In doing so, proteins are first separated electrophoretically and, after incubation in buffers containing an activity, which synthesises linear α-1,4-glucan chains (e.g. starch phosphorylase a) and its substrate (e.g. glucose-6-phosphate), the corresponding gels are coloured with iodine (Kimihiko et al., 1980, Analytical Biochemistry 108, 16-24).
10 Furthermore, branching enzymes in microbial organisms, such as the E. coli strain KV832 for example (Kiel et al., 1987 Mol. Gen. Genet 207: 294-301), which do not synthesise branched α-glucans, can be expressed. If an activity of a branching enzyme is introduced into the microbial organism due to the expression of a foreign gene in such strains (e.g. E. coli KV832), then the branching enzyme activity can be
15 demonstrated by treating colonies of these organisms with iodine vapour, for example. Colonies, which synthesise linear α-1,4-glucans, turn blue in this test, while colonies, which synthesise branched glucans by expressing an additional enzymatic activity of a branching enzyme, turn reddish-brown after treating with iodine vapour. It is also possible to express proteins in phosphoglucomutase mutants of E. coli to
20 identify a branching enzyme activity of appropriate proteins (Buettcher et al., 1999, Biochem. Biophys. Acta 1432, 406-412).
A further possibility of demonstrating branching enzyme activity of proteins is the use of a reaction stimulated by phosphorylase a and the subsequent separation of the products by means of thin film chromatography (Almstrupp et al., 2000, Analytical
25 Biochemistry 286, 297-300).
Branching enzyme activities can also be demonstrated with the help of the methods described in Guan and Preiss (1993, Plant Physiol. 102. 1269- 1273) and Kuriki et al. (1996, J. of Protein Chemistry 15, 305-313).

30 In conjunction with the present invention, the term "Class 3 branching enzyme" is to be understood as a branching enzyme, which has a higher degree of identity with the amino acid sequence shown in SEQ ID NO 4 than with that of the branching enzyme BE I from maize (GenBank Acc: D11081) or with that of the branching enzyme BE IIb

from maize (GenBank Acc: AF072725). Preferably, the Class 3 branching enzyme comes from starch-storing plants, particularly preferably from plant species of the genus *Solanum*, especially preferably from *Solanum tuberosum*.

5   In a further embodiment of the present invention, amino acid sequences coding Class 3 branching enzymes have an identity of at least 60% with the sequence shown in SEQ ID NO 4, in particular of at least 70%, preferably of at least 80% and particularly preferably of at least 90% and especially preferably of at least 95%.

10   According to the invention, Class 3 branching enzymes have an iso-amylase domain (Pfam acc.: Pf02922) and an alpha-amylase domain (Pfam acc: Pf00128). According to the invention, the iso-amylase domain and the alpha-amylase domain in amino acid sequences coding branching enzymes are separated from one another by the presence of further amino acids, which do not belong to these two domains.

15   Class 3 branching enzymes according to the invention are distinguished by the fact that the iso-amylase domain is separated from the alpha-amylase domain by a greater number of amino acids than the iso-amylase domain and the alpha-amylase domain of Class 1 and 2 branching enzymes.

Class 3 branching enzymes according to the invention are preferably distinguished

20   with regard to their amino acid sequence by the fact that they have at least 70, preferably at least 100, particularly preferably at least 130 and especially preferably at least 198 amino acids between the iso-amylase domain and the alpha-amylase domain. In a further embodiment of the present invention, in the case of an amino acid sequence coding a Class 3 branching enzyme, the C-terminal end of the iso-

25   amylase domain is separated from the N-terminal beginning of the alpha-amylase domain by 70 to 198, preferably by 100 to 198, particularly preferably by 130 to 198 and especially particularly preferably by 150 to 198 amino acids.

With the help of the Pfam database (Batemann et al., 2002, Nucleic Acids Research

30   30,   276-280;   accessible   via   http://www.sanger.ac.uk/Software/Pfam/, http://www.cgb.ki.se/Pfam/; http://pfam.jouy.inra.fr/ or http://pfam.wustl.edu/), it is possible for the person skilled in the art to determine whether amino acid sequences

already have known domains (e.g. an iso-amylase domain and/or an alpha-amylase domain).

Pfam is a database put together by experts, which classifies amino acid sequences into so-called families. Here, the assignment of an amino acid sequence to a family is

5   carried out on the basis of so-called domains, which are to be looked upon as functional and structural components of proteins. A domain is defined as a structural unit or a repeatedly occurring amino acid sequence unit, which can occur in proteins with widely different functions. Along with information relating to the amino acid sequence of known proteins, further knowledge (e.g. evidence of the enzymatic

10   activity, crystal structure data) is also used for the assignment of a protein to a family. Each family is assigned a name and an "accession" number (e.g. Name: Isoamylase_N, acc: PF02922). A constituent part of each family in the Pfam database is, amongst other things, a so-called "seed alignment". The "seed alignment" contains the amino acid sequences of representative proteins of a family.

15   Starting from "seed alignments", a so-called profile HMM ("profile Hidden Markov Model"; overview article in: Durbin et al., "Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids", Cambridge University Press, 1998, ISBN 0-521-62041-4) is produced using the HMMER 2 software (freely available under http://hmmer.wustl.edu/). The HMMs produced have names and are stored in the

20   Pfam database specifically for the correspondingly assigned domains. In contrast to classical, multiple "alignments" (e.g. produced using the Clustal W program or the Blossum62 algorithm), HMMs are based on a valid statistical theory (Bayes theory of conditional probability, Markoff chains) and enable an interrogation sequence (query) to be assigned to a family based on the use of position-specific evaluation matrices.

25   This enables an assignment to be made even when there are considerable differences in the amino acid sequences between the interrogation sequence (query) and a comparison sequence (e.g. amino acid sequence entry in a database).

The domain structure of the amino acid sequence concerned can be determined by

30   means of a comparison of the HMMs stored in the Pfam database with amino acid sequences, which are entered as a so-called interrogation sequence (query) (e.g. under http://hits.isb-sib.ch/cgi-bin/PFSCAN?).

In conjunction with the present invention, the term "iso-amylase domain" is to be understood to mean a Pfam iso-amylase domain (acc: Pf02922). At the same time, the HMM describing this Pfam iso-amylase domain is to be produced with the HMMER 2 [2.3.1] software, starting from a "seed alignment", which contains the

5 amino acid sequences shown in Table 1. In conjunction with the present invention, the "seed alignment" is produced by means of the ClustalW program (Thompson et al., Nucleic Acids Research 22 (1994), 4673-4680; see below). The following settings must be chosen to produce the appropriate HMMs: Build Method of HMM: hmmbuild –F HMM_ls, hmmcalibrate –seed 0 HMM_ls; Gathering cutoff: 2.3 2.3; Trusted cutoff:

10 2.3 2.2; Noise cutoff: 2.1 2.1). Further information for producing the HMM of the Pfam iso-amylase domain (acc: Pf02922) is given in Table 3.

In conjunction with the present invention, the term "alpha-amylase domain" is to be understood to mean a Pfam alpha-amylase domain (acc: Pf00128). At the same

15 time, the HMM describing this Pfam alpha-amylase domain is to be produced with the HMMER 2 [2.3.1] software, starting from a "seed alignment", which contains the amino acid sequences shown in Table 2. Here, the "seed alignment" is produced by means   of   HMM_simulated_annealing (http://www.psc.edu/general/software/packages/hmmer/manual/node11.html#SECTI

20 ON0032100000000000000). The following settings must be chosen to produce the appropriate HMM: Build Method of HMM: hmmbuild –F HMM_ls, hmmcalibrate –seed 0 HMM_ls; Gathering cutoff: -82.0 –82.0; Trusted cutoff: -81.7 –81.7; Noise cutoff: - 82.7 –82.7). Further information for producing the HMM of the Pfam alpha-amylase domain (acc: Pf00128) is given in Table 4.

25

In conjunction with the present invention, the term "Class 3 branching enzyme gene" is to be understood to mean a nucleic acid molecule (cDNA, DNA), which codes a Class 3 branching enzyme, preferably a Class 3 branching enzyme from starch- storing plants, particularly preferably from plant species of the genus *Solanum*,

30 especially preferably from *Solanum tuberosum*.

A preferred embodiment of the present invention relates to a genetically modified plant cell according to the invention or a genetically modified plant according to the

invention, wherein the genetic modification consists in the introduction of at least one foreign nucleic acid molecule into the genome of the plant cell or into the genome of the plant.

5   In this context, the term "genetic modification" means the introduction of homologous and/or heterologous foreign nucleic acid molecules into the genome of a plant cell or into the genome of a plant, wherein said introduction of these molecules leads to a reduction of the activity of a Class 3 branching enzyme.

The plant cells according to the invention or plants according to the invention are
10   modified with regard to their genetic information by the introduction of a foreign nucleic acid molecule. The presence or the expression of the foreign nucleic acid molecule leads to a phenotypic change. Here, "phenotypic" change means preferably a measurable change of one or more functions of the cells. For example, the genetically modified plant cells according to the invention and the genetically
15   modified plants according to the invention exhibit a reduction of the activity of a Class 3 branching enzyme due to the presence or on the expression of the introduced nucleic acid molecule.

In conjunction with the present invention, the term "foreign nucleic acid molecule" is
20   understood to mean such a molecule that either does not occur naturally in the corresponding wild type plant cells that have not been genetically modified, or that does not occur naturally in the concrete spatial arrangement in wild type plant cells that have not been genetically modified, or that is localised at a place in the genome of the wild type plant cell at which it does not occur naturally. Preferably, the foreign
25   nucleic acid molecule is a recombinant molecule, which consists of different elements, the combination or specific spatial arrangement of which does not occur naturally in vegetable cells.

In principle, the foreign nucleic acid molecule can be any nucleic acid molecule, which effects a reduction of the activity of a Class 3 branching enzyme in the plant
30   cell or plant.

In conjunction with the present invention, the term "genome" is to be understood to mean the totality of the genetic material present in a vegetable cell. It is known to the

person skilled in the art that, as well as the cell nucleus, other compartments (e.g. plastids, mitochondrions) also contain genetic material.

In a further embodiment, the plant cells according to the invention and the plants
5   according to the invention are characterised in that the foreign nucleic acid molecule codes a Class 3 branching enzyme, preferably a Class 3 branching enzyme from starch-storing plants, particularly preferably from plants of a species of the genus *Solanum*, especially preferably from *Solanum tuberosum*.

10 In a particularly preferred embodiment, the foreign nucleic acid molecule codes a Class 3 branching enzyme with the amino acid sequence specified in SEQ ID NO 4.

A large number of techniques are available for the introduction of DNA into a vegetable host cell. These techniques include the transformation of vegetable cells
15 with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as the transformation medium, the fusion of protoplasts, injection, the electroporation of DNA, the introduction of DNA by means of the biolistic approach as well as other possibilities.

The use of agrobacteria-mediated transformation of plant cells has been intensively
20 investigated and adequately described in EP 120516; Hoekema, IN: The Binary Plant Vector System Offsetdrukkerij Kanters B.V., Alblasserdam (1985), Chapter V; Fraley et al., Crit. Rev. Plant Sci. 4, 1-46 and by An et al. EMBO J. 4, (1985), 277-287. For the transformation of potato, see Rocha-Sosa et al., EMBO J. 8, (1989), 29-33, for example.

25

The transformation of monocotyledonous plants by means of vectors based on agrobacterium transformation has also been described (Chan et al., Plant Mol. Biol. 22, (1993), 491-506; Hiei et al., Plant J. 6, (1994) 271-282; Deng et al, Science in China 33, (1990), 28-34; Wilmink et al., Plant Cell Reports 11, (1992), 76-80; May et
30 al., Bio/Technology 13, (1995), 486-492; Conner and Domisse, Int. J. Plant Sci. 153 (1992), 550-555; Ritchie et al, Transgenic Res. 2, (1993), 252-265). An alternative system to the transformation of monocotyledonous plants is transformation by means of the biolistic approach (Wan and Lemaux, Plant Physiol. 104, (1994), 37-48; Vasil

et al., Bio/Technology 11 (1993), 1553-1558; Ritala et al., Plant Mol. Biol. 24, (1994), 317-325; Spencer et al., Theor. Appl. Genet. 79, (1990), 625-631), protoplast transformation, electroporation of partially permeabilised cells and the introduction of DNA by means of glass fibres. In particular, the transformation of maize has been
5   described in the literature many times (cf. e.g. WO95/06128, EP0513849, EP0465875, EP0292435; Fromm et al., Biotechnology 8, (1990), 833-844; Gordon-Kamm et al., Plant Cell 2, (1990), 603-618; Koziel et al., Biotechnology 11 (1993), 194-200; Moroc et al., Theor. Appl. Genet. 80, (1990), 721-726).

The successful transformation of other types of cereal has also already been
10   described, for example for barley (Wan and Lemaux, see above; Ritala et al., see above; Krens et al., Nature 296, (1982), 72-74) and for wheat (Nehra et al., Plant J. 5, (1994), 285-297). All the above methods are suitable within the framework of the present invention.

15   Amongst other things, the plant cells according to the invention and the plants according to the invention can be differentiated from wild type plant cells and wild type plants respectively in that they contain a foreign nucleic acid molecule, which does not occur naturally in wild type plant cells or wild type plants, or in that such a molecule is present integrated at a place in the genome of the plant cell according to
20   the invention or in the genome of the plant according to the invention at which it does not occur in wild type plant cells or wild type plants, i.e. in a different genomic environment. Furthermore, plant cells according to the invention and plants according to the invention of this type differ from wild type plant cells and wild type plants respectively in that they contain at least one copy of the foreign nucleic acid molecule
25   stably integrated within their genome, possibly in addition to naturally occurring copies of such a molecule in the wild type plant cells or wild type plants. If the foreign nucleic acid molecule(s) introduced into the plant cells according to the invention or into the plants according to the invention is (are) additional copies of molecules already occurring naturally in the wild type plant cells or wild type plants respectively,
30   then the plant cells according to the invention and the plants according to the invention can be differentiated from wild type plant cells or wild type plants respectively in particular in that this additional copy or these additional copies is (are) localised at places in the genome at which it does not occur (or they do not occur) in

wild type plant cells or wild type plants. This can be verified, for example, with the help of a Southern blot analysis.

Furthermore, the plant cells according to the invention and the plants according to the invention can preferably be differentiated from wild type plant cells or wild type

5   plants respectively by at least one of the following characteristics: If the foreign nucleic acid module that has been introduced is heterologous with respect to the plant cell or plant, then the plant cells according to the invention or plants according to the invention have transcripts of the introduced nucleic acid molecules. These can be verified, for example, by Northern blot analysis or by RT-PCR (Reverse

10 Transcription Polymerase Chain Reaction). Plant cells according to the invention and plants according to the invention, which express an antisense and/or an RNAi transcript, can be verified, for example, with the help of specific nucleic acid probes, which are complimentary to the RNA (occurring naturally in the plant cell), which is coding for the protein.

15 If the foreign nucleic acid module that has been introduced is homologous with respect to the plant cell or plant, the plant cells according to the invention or plants according to the invention can be differentiated from wild type plant cells or wild type plants respectively due to the additional expression of the introduced foreign nucleic acid molecule, for example. The plant cells according to the invention and the plants

20 according to the invention preferably contain (sense and/or antisense) transcripts of the foreign nucleic acid molecules. This can be demonstrated by Northern blot analysis, for example, or with the help of so-called quantitative PCR.

In a special embodiment, the plant cells according to the invention and the plants

25 according to the invention are transgenic plant cells or transgenic plants respectively.

In a further embodiment, the present invention relates to plant cells according to the invention and plants according to the invention wherein the foreign nucleic acid molecule is chosen from the group consisting of

30 a)   Nucleic acid molecules, which code a protein with the amino acid sequence given under Seq ID NO 4;

b)   Nucleic acid molecules, which code a protein, the amino acid sequence of which has an identity of at least 50% with the amino acid sequence given under SEQ ID NO: 4;

c)   Nucleic acid molecules, which include the nucleotide sequence shown under Seq ID NO 3 or a complimentary sequence;

d)   Nucleic acid molecules, the nucleic acid sequence of which has an identity of at least 50% with the nucleic acid sequences described under a) or c);

e)   Nucleic acid molecules, which hybridise with at least one strand of the nucleic acid molecules described under a) or c) under stringent conditions;

f)   Nucleic acid molecules, the nucleotide sequence of which deviates from the sequence of the nucleic acid molecules identified under a), b), c), d), e) or f) due to the degeneration of the genetic code; and

g)   Nucleic acid molecules, which represent fragments, allelic variants and/or derivatives of the nucleic acid molecules identified under a), b), c), d), e) or f).

A further embodiment of the present invention relates to plant cells according to the invention and plants according to the invention wherein the foreign nucleic acid molecule is chosen from the group consisting of

a)   Nucleic acid molecules, which code at least one antisense RNA, which effects a reduction in the expression of at least one endogenous gene, which codes a Class 3 branching enzyme;

b)   Nucleic acid molecules, which by means of a co-suppression effect lead to the reduction in the expression of at least one endogenous gene, which codes a Class 3 branching enzyme;

c)   Nucleic acid molecules, which code at least one ribozyme, which splits specific transcripts of at least one endogenous gene, which codes a Class 3 branching enzyme;

d)   Nucleic acid molecules, which simultaneously code at least one antisense RNA and at least one sense RNA, wherein the said antisense RNA and the said sense RNA form a double-stranded RNA molecule, which effects a reduction in the expression of at least one endogenous gene, which codes a Class 3 branching enzyme (RNAi technology);

e) Nucleic acid molecules introduced by means of in vivo mutagenesis, which lead to a mutation or an insertion of a heterologous sequence in at least one endogenous gene coding a Class 3 branching enzyme, wherein the mutation or insertion effects a reduction in the expression of a gene coding a Class 3 branching enzyme or results in the synthesis of inactive Class 3 branching enzymes;

f) Nucleic acid molecules, which code an antibody, wherein the antibody results in a reduction in the activity of a Class 3 branching enzyme due to the bonding to a Class 3 branching enzyme.

g) DNA molecules, which contain transposons, wherein the integration of these transposons leads to a mutation or an insertion in at least one endogenous gene coding a Class 3 branching enzyme, which effects a reduction in the expression of at least one gene coding a Class 3 branching enzyme, or results in the synthesis of inactive Class 3 branching enzymes; and/or

h) T-DNA molecules, which, due to insertion in at least one endogenous gene coding a Class 3 branching enzyme, effect a reduction in the expression of at least one gene coding a Class 3 branching enzyme, or result in the synthesis of inactive Class 3 branching enzyme.

The plant cells according to the invention and plants according to the invention can be manufactured by different methods known to the person skilled in the art. These include, for example, the expression of a corresponding antisense RNA or of a double-stranded RNA construct, the provision of molecules or vectors, which impart a cosuppression effect, the expression of a correspondingly constructed ribozyme that splits specific transcripts, which code a Class 3 branching enzyme, or so-called "in vivo mutagenesis". Furthermore, the reduction of the Class 3 branching enzyme activity in plant cells and plants can also be brought about by the simultaneous expression of sense and antisense RNA molecules of the respective target gene to be repressed, preferably of the Class 3 branching enzyme gene.

In addition to this, it is known that *in planta* the formation of double-stranded RNA molecules of promoter sequences can lead *in trans* to methylation and transcriptional inactivation of homologous copies of this promoter (Mette et al., EMBO J. 19, (2000), 5194-5201).

A further possible way in which to reduce the enzymatic activity of proteins in plant cells or plants is the so-called immunomodulation method. It is known that an *in planta* expression of antibodies, which specifically recognise a vegetable protein, results in a reduction of the activity of the proteins concerned in appropriate plant
5   cells due to the formation of a protein antibody complex (Conrad and Manteufel, Trends in Plant Science 6, (2001), 399-402; De Jaeger et al., Plant Molecular Biology 43, (2000), 419-428; Jobling et al., Nature Biotechnology 21, (2003), 77-80).

All these methods are based on the introduction of a foreign or of several foreign nucleic acid molecules into the genome of plant cells or plants and are therefore
10 basically suitable for manufacturing plant cells according to the invention and plants according to the invention.

For inhibiting the expression of genes by means of antisense or cosuppression technology, a DNA molecule can be used, for example, which includes the whole
15 coding sequence for a Class 3 branching enzyme, including any existing flanking sequences, as well as DNA molecules, which include only parts of the coding sequence, whereby these parts must be long enough to produce an antisense effect or a cosuppression effect respectively in the cells. In general, sequences up to a minimum length of 21 bp, preferably a minimum length of at least 100 bp, particularly
20 preferably of at least 500 bp are suitable. For example, the DNA molecules have a length of 21-100 bp, preferably of 100-500 bp, particularly preferably over 500 bp.

The use of DNA sequences, which have a high degree of identity with the endogenous sequences occurring in the plant cells and which code Class 3
25 branching enzymes, is also suitable for antisense or cosuppression approaches. The minimum identity should be greater than ca. 65%, preferably greater than 80%. The use of sequences with identities of at least 90%, in particular between 95% and 100%, is to be preferred. The meaning of the term "identity" will be defined elsewhere.
30

Furthermore, the use of introns, i.e. of non-coding areas of genes, which code for Class 3 branching enzymes, is also conceivable for achieving an antisense or a cosuppression effect.

The use of intron sequences for inhibiting the gene expression of genes, which code for starch biosynthesis proteins, has been described in the international patent applications WO97/04112, WO97/04113, WO98/37213, WO98/37214.

5   The person skilled in the art knows how to achieve an antisense and a cosuppression effect. For example, the method of cosuppression inhibition has been described in Jorgensen (Trends Biotechnol. 8 (1990), 340-344), Niebel et al., (Curr. Top. Microbiol. Immunol. 197 (1995), 91-103), Flavell et al. (Curr. Top. Microbiol. Immunol. 197 (1995), 43-46), Palaqui and Vaucheret (Plant. Mol. Biol. 29 (1995),
10 149-159), Vaucheret et al., (Mol. Gen. Genet. 248 (1995), 311-317), de Borne et al. (Mol. Gen. Genet. 243 (1994), 613-621).

The expression of ribozymes for reducing the activity of particular enzymes in cells is also known to the person skilled in the art, and is described, for example, in EP-B1
15 0321201. The expression of ribozymes in vegetable cells has been described, for example, in Feyter et al. (Mol. Gen. Genet. 250, (1996), 329-338).

The reduction of the activity of a Class 3 branching enzyme in plant cells according to the invention and plants according to the invention can also be brought about by the
20 simultaneous expression of sense and antisense RNA molecules (RNAi technology) of the respective target gene to be repressed, preferably of the Class 3 branching enzyme gene.

This can be achieved, for example, by the use of chimeric constructs, which contain "inverted repeats" of the respective target gene or parts of the target gene. In this
25 case, the generic constructs code for sense and antisense RNA molecules of the respective target gene. Sense and antisense RNA are synthesised simultaneously in planta as an RNA molecule, wherein sense and antisense RNA are separated from one another by a spacer, and are able to form a double-stranded RNA molecule.

It has been shown that the introduction of inverted repeat DNA constructs into the
30 genome of plant cells or plants is a very effective method of repressing the genes corresponding to the inverted repeat DNA constructs (Waterhouse et al., Proc. Natl. Acad. Sci. USA 95, (1998), 13959-13964; Wang and Waterhouse, Plant Mol. Biol. 43, (2000), 67-82; Singh et al., Biochemical Society Transactions Vol. 28 part 6

(2000), 925- 927; Liu et al., Biochemical Society Transactions Vol. 28 part 6 (2000), 927-929); Smith et al., (Nature 407, (2000), 319-320; international patent application WO99/53050 A1). Sense and antisense sequences of the target gene or the target genes can also be expressed separately from one another by means of similar or

5   different promoters (Nap, J-P et al, 6th International Congress of Plant Molecular Biology, Quebec, 18th-24th June, 2000; Poster S7-27, Presentation Session S7).

The reduction of the activity of a Class 3 branching enzyme in plant cells according to the invention or plants according to the invention can therefore also be achieved by

10 producing double-stranded RNA molecules. In this regard, "inverted repeats" of DNA molecules of Class 3 branching enzyme genes or cDNAs are preferably introduced into the genome of plants, wherein the DNA molecules (Class 3 branching enzyme gene or cDNA or fragments of this gene or cDNA) to be transcribed are under the control of a promoter, which controls the expression of said DNA molecules.

15

In addition to this, it is known that the formation of double-stranded RNA molecules from promoter DNA molecules in plants in trans can lead to methylation and transcriptional inactivation of homologous copies of these promoters, which are to be referred to in the following as target promoters (Mette et al., EMBO J. 19, (2000),

20 5194-5201).

It is therefore possible to reduce the gene expression of a particular target gene (e.g. branching enzyme Class 3 gene), which is naturally under the control of this target promoter, by deactivating the target promoter.

This means that, in this case, the DNA molecules, which include the target promoters

25 of the genes to be repressed (target genes), in contrast to the original function of promoters in plants, are not used as control elements for the expression of genes or cDNAs, but are themselves used as transcribable DNA molecules.

For the production of double-stranded target promoter RNA molecules in planta, which can occur there as RNA hairpin molecules, constructs are preferably used,

30 which contain the "inverted repeats" of the target promoter DNA molecules, wherein the target promoter DNA molecules are under the control of a promoter, which controls the gene expression of said target promoter DNA molecules. These constructs are subsequently introduced into the genome of plants. The expression of

the "inverted repeats" of said target promoter DNA molecules *in planta* leads to the formation of double-stranded target promoter RNA molecules (Mette et al., EMBO J. 19, (2000), 5194-5201). The target promoter can be inactivated by this means.

The reduction of the activity of a Class 3 branching enzyme in plant cells according to

5   the invention and plants according to the invention can therefore also be achieved by the production of double-stranded RNA molecules of promoter sequences of Class 3 branching enzyme genes. In this regard, "inverted repeats" of promoter DNA molecules of Class 3 branching enzyme genes are preferably introduced into the genome of plants, wherein the target promoter DNA molecules (promoter of a Class

10   3 branching enzyme gene) to be transcribed are under the control of a promoter, which controls the expression of said target promoter DNA molecules.

For inhibiting the expression of genes by means of the simultaneous expression of sense and antisense RNA molecules (RNAi technology), a DNA molecule can be

15   used, for example, which includes the whole coding sequence for a Class 3 branching enzyme, including any existing flanking sequences, as well as DNA molecules, which include only parts of the coding sequence, whereby these parts must be long enough to produce a so-called RNAi effect in the cells. In general, sequences with a minimum length of 40 bp, preferably a minimum length of at least

20   100 bp, particularly preferably of at least 500 bp are suitable. For example, the DNA molecules have a length of 21-100 bp, preferably of 100-500 bp.

The use of DNA sequences, which have a high degree of identity with the endogenous sequences occurring in the plant cells and which code Class 3

25   branching enzymes, is also suitable for the simultaneous expression of sense and antisense RNA molecules (RNAi technology). The minimum identity should be greater than ca. 65%, preferably greater than 80%. The use of sequences with identities of at least 90%, in particular between 95% and 100%, is to be particularly preferred.

30

Furthermore, the reduction of the activity of a Class 3 branching enzyme in plant cells according to the invention and plants according to the invention can also be achieved by so-called "in vivo mutagenesis", in which a hybrid RNA-DNA oligonucleotide

("Chimeroplast") is introduced into plant cells (Kipp, P.B. et al., Poster Session at the "5th International Congress of Plant Molecular Biology, 21st-27th September 1997, Singapore; R. A. Dixon and C.J. Arntzen, meeting report on "Metabolic Engineering in Transgenic Plants", Keystone Symposia, Copper Mountain, CO, USA, TIBTECH

5   15, (1997), 441-447; international patent application WO 9515972; Kren et al., Hepatology 25, (1997), 1462-1468; Cole-Strauss et al., Science 273, (1996), 1386-1389; Beetham et al., 1999, PNAS 96, 8774-8778).

A part of the DNA components of the RNA-DNA oligonucleotide is homologous to a nucleic acid sequence of an endogenous Class 3 branching enzyme gene, but, in

10   comparison with the nucleic acid sequence of a Class 3 branching enzyme gene, it has a mutation or contains a heterologous region, which is surrounded by the homologous regions.

By base pairing of the homologous regions of the RNA-DNA oligonucleotide and the endogenous nucleic acid molecule followed by homologous recombination, the

15   mutation or heterologous region contained in the DNA components of the RNA-DNA oligonucleotide can be transferred into the genome of a plant cell. This leads to the reduction of the activity of one or more Class 3 branching enzymes.

The person skilled in the art knows that he can achieve the activity of Class 3

20   branching enzymes by the expression of non-functional derivatives, in particular transdominant mutants, of such proteins, and/or by the expression of antagonists/inhibitors of such proteins.

Antagonist/inhibitors of such proteins include, for example, antibodies, antibody fragments or molecules with similar bonding characteristics. For example, a

25   cytoplasmatic scFv antibody has been used to modulate the activity of the phytochrome A protein in genetically modified tobacco plants (Owen, Bio/Technology 10 (1992), 790-4; Review: Franken, E, Teuschel, U. and Hain, R., Current Opinion in Biotechnology 8, (1997), 411-416; Whitelam, Trends Plant Sci. 1 (1996), 268-272; Conrad and Manteufel, Trends in Plant Science 6, (2001), 399-402; De Jaeger et al.,

30   Plant Molecular Biology 43, (2000), 419-428). The reduction of the activity of a branching enzyme in potato plants by expressing a specific antibody has been described by Jobling et al. (Nature Biotechnology 21, (2003), 77-80). Here, the

antibody was provided with a plastidiary target sequence so that the inhibition of proteins localised in plastids was guaranteed.

In conjunction with the present invention, plant cells and plants according to the
5   invention can also be manufactured by the use of so-called insertion mutagenesis (overview article: Thorneycroft et al., 2001, Journal of experimental Botany 52 (361), 1593-1601). Insertion mutagenesis is to be understood to mean particularly the insertion of transposons or so-called transfer DNA (T-DNA) into a gene coding for a Class 3 branching enzyme, whereby, as a result of which, the activity of a Class 3
10   branching enzyme in the cell concerned is reduced.

The transposons can be both those that occur naturally in the cell (endogenous transposons) and also those that do not occur naturally in said cell but are introduced into the cell (heterologous transposons) by means of genetic engineering methods,
15   such as transformation of the cell, for example. Changing the expression of genes by means of transposons is known to the person skilled in the art. An overview of the use of endogenous and heterologous transposons as tools in plant biotechnology is presented in Ramachandran and Sundaresan (2001, Plant Physiology and Biochemistry 39, 234-252). The possibility of identifying mutants in which specific
20   genes have been inactivated by transposon insertion mutagenesis is presented in an overview by Maes et al. (1999, Trends in Plant Science 4 (3), 90-96). The production of rice mutants with the help of endogenous transposons is described by Hirochika (2001, Current Opinion in Plant Biology 4, 118-122). The identification of maize genes with the help of endogenous retrotransposons is presented, for example, by
25   Hanley et al. (2000, The Plant Journal 22 (4), 557-566). The possibility of manufacturing mutants with the help of retrotransposons and methods of identifying mutants are described by Kumar and Hirochika (2001, Trends in Plant Science 6 (3), 127-134). The activity of technological transposons in different species has been described both for dicotyledonous and for monocotyledonous plants: e.g. for rice
30   (Greco et al., 2001, Plant Physiology 125, 1175-1177; Liu et al., 1999, Molecular and General Genetics 262, 413-420; Hiroyuki et al., 1999, The Plant Journal 19 (5), 605-613; Jeon und Gynheung, 2001, Plant Science 161, 211-219), barley (2000, Koprek et al., The Plant Journal 24 (2), 253-263) Arabidopsis thaliana (Aarts et al., 1993,

Nature 363, 715-717, Schmidt und Willmitzer, 1989, Molecular and General Genetics 220, 17-24; Altmann et al., 1992, Theoretical and Applied Gentics 84, 371-383; Tissier et al., 1999, The Plant Cell 11, 1841-1852), tomato (Belzile und Yoder, 1992, The Plant Journal 2 (2), 173-179) and potato (Frey et al., 1989, Molecular and 5   General Genetics 217, 172-177; Knapp et al., 1988, Molecular and General Genetics 213, 285-290).

Basically, the plant cells according to the invention and the plants according to the invention can be manufactured both with the help of homologous and heterologous 10   transposons, whereby the use of homologous transposons is also to be understood to mean those, which are naturally present in the corresponding wild type plant genome.

T-DNA insertion mutagenesis is based on the fact that certain sections (T-DNA) of Ti 15   plasmids from *Agrobacterium* can integrate into the genome of vegetable cells. The place of integration in the vegetable chromosome is not defined, but can take place at any point. If the T-DNA integrates into a part of the chromosome, which constitutes a gene function, then this can lead to a change in the gene expression and thus also to a change in the activity of a protein coded by the gene concerned. In particular, the 20   integration of a T-DNA into the coded area of a protein often leads to the corresponding protein no longer being able to be synthesised at all, or no longer synthesised in active form, by the cell concerned. The use of T-DNA insertions for producing mutants is described, for example, for *Arabidopsis thaliana* (Krysan et al., 1999, The Plant Cell 11, 2283-2290; Atipiroz-Leehan and Feldmann, 1997, Trends in 25   genetics 13 (4), 152-156; Parinov and Sundaresan, 2000, Current Opinion in Biotechnology 11, 157-161) and rice (Jeon and An, 2001, Plant Science 161, 211-219; Jeon et al., 2000, The Plant Journal 22 (6), 561-570). Methods for identifying mutants, which have been produced with the help of T-DNA insertion mutagenesis, are described, amongst others, by Young et al., (2001, Plant Physiology 125, 513-30   518), Parinov et al. (1999, The Plant cell 11, 2263-2270), Thorneycroft et al. (2001, Journal of Experimental Botany 52, 1593-1601), and McKinney et al. (1995, The Plant Journal 8 (4), 613-622).

T-DNA mutagenesis is basically suitable for the production of the plant cells and plants according to the invention, which have a reduced activity of a Class 3 branching enzyme.

5   Surprisingly, it has been found that plant cells according to the invention and plants according to the invention synthesise a modified starch in comparison with starch of corresponding wild type plant cells or wild type plants that have not been genetically modified.

10   The plant cells according to the invention and plants according to the invention synthesise a modified starch, which in its physical-chemical characteristics, in particular the amylose content or the amylose/amylopectin ratio, the degree of branching, the average chain length, the side chain distribution, the viscosity behaviour, the gelling strength, the starch grain size and/or the starch grain
15   morphology, is changed in comparison with the synthesised starch in wild type plant cells or plants, so that this is better suited for special applications.

It was surprisingly found that plant cells or plants of the invention synthesize a modified starch having decreased phosphate content. So far known plants with a
20   reduced activity of a branching enzyme (Class 1 and/or Class 2) did show an increased phosphate content.

The present invention therefore also includes plant cells according to the invention and plants according to the invention, which synthesise a modified starch.
25
In a preferred embodiment of the invention, the plant cells according to the invention or the plant according to the invention synthesize a starch with a decreased phosphate content in comparison with corresponding starch isolated from wild type plant cells or wild type plants that have not been genetically modified. Preferably the
30   plant cells according to the invention or the plants according to the invention synthesize a starch having a total phosphate content that is decreased by at least 10%, more preferably by at least 15% and particular preferably by at least 20% in comparison with starch isolated from corresponding wild type plant cells or wild type

plants that have not been genetically modified. Especially preferably the total phosphate content of starch isolated from plant cells of the invention or plants of the invention is decreased by 14% to 22% in comparison with starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically

5   modified.

In respect with C-6-phoaphate content the plant cells according to the invention or the plants according to the invention synthesize a starch having a C-6-phosphate content that is decreased by at least 15%, more preferably by at least 19% and

10   particular preferably by at least 25% in comparison with starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified. Especially preferably the C-6-phoaphate content of starch isolated from plant cells of the invention or plants of the invention is decreased by 15%% to 27% in comparison with starch isolated from corresponding wild type plant cells or wild type

15   plants that have not been genetically modified.

Methods for the determination of total phosphate or C-6-phosphate content in starches are well known by a person skilled in the art. Preferred methods for the determination of total or C-6-phosphate content in starches to be used in combination

20   with the present invention are described below in the section "general methods" (Starch analysis, e) Analysis of the side-chain distribution of the amylopectin by means of ion-exchange chromatography).

In a further prefered embodiment embodiment of the invention, the plant cells

25   according to the invention or the plants according to the invention synthesize a starch which has has an altered viscosity behaviour in comparison with starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified. Plant cells of the invention or plants of the invention synthesize a starch which has a decreased maximum viscosity, a decreased minimum viscosity and/or a

30   decreased final viscosity in comparison with starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified.
The maximum viscosity of starch isolated from plant cells of the invention or plants of the invention is preferably decreased by at least 8% and more preferably by at least

16% in comparison with starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified. Especially preferably the maximum viscosity of starch isolated from plant cells of the invention or plants of the invention is decreased by 8% to 16%_in comparison with starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified.

5

The minimum viscosity of starch isolated from plant cells of the invention or plants of the invention is preferably decreased by at least 10%, more preferably by at least 15% and particularly preferably by at lest 25% in comparison with starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified. Especially preferably the minimum viscosity of starch isolated from plant cells of the invention or plants of the invention is decreased by 15% to 25% in comparison with starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified.

10

The final viscosity of starch isolated from plant cells of the invention or plants of the invention is preferably decreased by at least 5% and more preferably by at least 10% in comparison with starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified. Particularly preferably the minimum viscosity of starch isolated from plant cells of the invention or plants of the invention is decreased by 5% to 10% in comparison with starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified.

15

20

It has further been found, that starch isolated from plant cells of the invention or plants of the invention shows an increased gelling strength in comparison with starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically_modified.

25

The present invention therefore also comprises plant cells of the invention or plants of the invention that synthesize a starch with an increased gel strength in comparison with starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified. Preferably plant cells of the invention or plants of the invention synthesize a starch which shows a gel strength which is increased by at

30

least 20%, more preferably by at least 30% and particular preferably by at least 35% in comparison with starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified. Especially preferably the gel strength of starch isolated from plant cells of the invention or plants of the invention is

5   increased by 27% to 38% in comparison with starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified.

Methods for the determination of viscosity behaviour or gelling properties of starches are well known by a person skilled in the art. Preferred methods for the determination

10   of viscosity behaviour or gelling properties of starches to be used in combination with the present invention are described below in the section "general methods".

Furthermore it was surprisingly found that starch, isolated from plant cells of the invention or plants of the invention shows an altered side chain distribution pattern in

15   the amylopectin fraction in comparison with the amylopectin fraction from starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified.

In a further embodiment of the invention, plant cells according to the invention or the

20   plants according to the invention synthesize a starch with an altered short-side-chain distribution pattern in the amylopectin fraction in comparison with the amylopectin fraction from starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified. Preferably plant cells according to the invention or the plants according to the invention synthesize a starch wherein the

25   short-side-chains in the amylopectin fraction having a degree of polymerization (DP) of 6 and/or a DP of 7 is increased in comparison with the amylopectin fraction from starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified. More preferably the amylopectin fraction of starch isolated form plant cells according to the invention or plants according to the

30   invention synthesize a starch wherein short-side-chains with a DP 6 is increased by at least 15%, particularly preferably by at least 20%, especially particularly by at least 25% and/or the short-side-chains with a DP 7 are increased by at least 2%, particularly preferably by at least 4%, especially preferably by at least 8% in

comparison with the amylopectin fraction from starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified.

In a further preferred embodiment of the invention the plant cells according to the invention or the plants according to the invention synthesize a starch wherein the

5   short-side-chains of DP 6 in the amylopectin fraction is increased by 17% to 29% and/or the side chains of DP 7 in the amylopectin fraction is increased by 2% to 9% in comparison with the amylopectin fraction from starch isolated from corresponding wild type plant cells or wild type plants that have not been genetically modified.

10   In conjunction with the present invention, the term "short-side-chain" shall mean alpha-1,6-linked side-chains in the starch molecule having a degree of polymerization between DP 6 and DP 34.

Methods for the quantification of short-side-chains having a specified DP in the

15   amylopectin fraction are well known by the person skilled in the art. Preferred methods for the quantification of side-chains having a specified DP, suitable to be used in combination with the present invention are described below in the section "general methods (Analysis of the side-chain distribution of the amylopectin by means of ion-exchange chromatography).

20

Furthermore it was found that the amylopectin fraction of starch, isolated from the plant cells according to the invention or the plants according to the invention shows an altered total-side-chain distribution.

25   Therefore, further embodiments of the present invention are the plant cells according to the invention or the plants according to the invention which synthesize a starch wherein the groups of total-side-chains in the amylopectin fraction characterized by the following ranges:

a) DP up to 11,

30   b) DP 12 to DP 19,

c) DP 20 to Dp 25 and/or

d) DP 26 to DP 31

is/are increased and/or the groups of total-side-chains in the amylopectin fraction characterized by the following ranges:

    a) DP 38 to DP 43

    b) DP 44 to DP 49

5   c) DP 50 to DP 56

    d) DP 57 to DP 62 and/or

    e) DP 63 to DP 123

Is/are decreased in comparison with the amylopectin fraction from starch isolated from corresponding wild type plant cells or wild type plants that have not been

10 genetically modified.

In conjunction with the present invention, the term "total-side-chains" shall mean alpha-1,6-linked side-chains in the starch molecule having a degree of polymerization up to DP 123. A group of total-side-chains consists of all side-chains spanning a

15 defined DP range (e.g. DP up to 11, DP 12 to DP 19, DP 20 to Dp 25, DP 26 to DP 31, DP 38 to DP 43, DP 44 to DP 49, DP 50 to DP 56, DP 57 to DP 62, DP 63 to DP 123).

Methods for the quantification of groups of total-side-chains spanning ranges of side-

20 chains with a specified DP in the amylopectin fraction are well known by the person skilled in the art. Preferred methods for the quantification groups of total-side-chains, suitable to be used in combination with the present invention are described below in example 5d).

25 Further embodiments of the invention are the plant cells according to the invention or the plants according to the invention which synthesize a starch having a decreased peak onset Temperature (T_0), a decreased peak temperature (T Peak) and an increased delta H (dH) when analyzed by differential scanning calorimetrie (DSC) in comparison to starch isolated from corresponding wild type plant cells or wild type

30 plants that have not been genetically modified.

Methods for the analysis of starch by DSC are well known by a person skilled in the art. Prefered Methods for DSC analysis suitable to be used in combination with the

present invention are described below in the section "general methods" (DSC-analysis ("Differential Scanning Calorimetry").

Furthermore, genetically modified plants, which contain the plant cells according to
5   the invention, are also the subject matter of the invention. Plants of this type can be produced from plant cells according to the invention by regeneration.
In principle, the plants according to the invention can be plants of any plant species, i.e. both monocotyledonous and dicotyledonous plants. Preferably they are useful plants, i.e. plants, which are cultivated by people for the purposes of food or for
10   technical, in particular industrial purposes.

In a further preferred embodiment, the plant according to the invention is a starch-storing plant.

15   In a further preferred embodiment, the present invention relates to starch-storing plants according to the invention of the genus *Solanum*, in particular *Solanum tuberosum*.

The term "starch-storing plants" includes all plants with starch-storing plant parts
20   such as, for example, maize, rice, wheat, rye, oat, barley, cassava, potato, sago, mung bean, pea or sorghum. Preferred starch-storing plant parts are, for example, tubers, storage roots and grains containing an endosperm; tubers are particularly preferred.

25   In conjunction with the present invention, the term "potato plant" or "potato" means plant species of the genus *Solanum*, in particular tuber-producing species of the genus *Solanum* and especially *Solanum tuberosum*.

The present invention also relates to propagation material of plants according to the
30   invention containing a plant cell according to the invention.

Here, the term "propagation material" includes those constituents of the plant that are suitable for producing offspring by vegetative or sexual means. Cuttings, callus

cultures, rhizomes or tubers, for example, are suitable for vegetative propagation. Other propagation material includes, for example, fruits, seeds, seedlings, protoplasts, cell cultures, etc. Preferably, the propagation material is seeds and particularly preferably tubers.

5

In a further embodiment, the present invention relates to harvestable plant parts of plants according to the invention such as fruits, storage roots, roots, blooms, buds, shoots or stems, preferably seeds or tubers, wherein these harvestable parts contain at least one plant cell according to the invention.

10

Furthermore, the present invention also relates to a method for the manufacture of a plant according to the invention, wherein

a)   a plant cell is genetically modified, whereby the genetic modification leads to the reduction of the activity of a Class 3 vegetable branching enzyme in

15   comparison with corresponding wild type plant cells that have not been genetically modified;

b)   a plant is regenerated from plant cells from Step a); and

c)   if necessary, further plants are produced with the help of the plants according to Step b).

20

The genetic modification introduced into the plant cell according to Step a) can basically be any type of genetic modification, which leads to the reduction of the activity of a Class 3 branching enzyme.

25   The regeneration of the plants according to Step (b) can be carried out using methods known to the person skilled in the art (e.g. described in "Plant Cell Culture Protocols", 1999, edt. by R.D. Hall, Humana Press, ISBN 0-89603-549-2).

The production of further plants according to Step (c) of the method according to the

30   invention can be carried out, for example, by vegetative propagation (for example using cuttings, tubers or by means of callus culture and regeneration of whole plants) or by sexual propagation. Here, sexual propagation preferably takes place under

controlled conditions, i.e. selected plants with particular characteristics are crossed and propagated with one another.

In a preferred embodiment of the method according to the invention, the genetic
5   modification consists in the introduction of a foreign nucleic acid molecule into the genome of the plant cell, wherein the presence or the expression of said foreign nucleic acid molecule leads to a reduced activity of a Class 3 branching enzyme in the cell.

10 The statements made in conjunction with plant cells according to the invention and plants according to the invention apply with regard to the "introduction of a foreign nucleic acid molecule".

In a further preferred embodiment, the method according to the invention is used for
15 producing potato plants according to the invention.

In a further preferred embodiment of the method according to the invention, the foreign nucleic acid molecule is chosen from the group consisting of

a)   Nucleic acid molecules, which code a protein with the amino acid sequence
20   given under Seq ID NO 4;

b)   Nucleic acid molecules, which code a protein, the amino acid sequence of which has an identity of at least 50% with the amino acid sequence given under SEQ ID NO: 4;

c)   Nucleic acid molecules, which include the nucleotide sequence shown under
25   Seq ID NO. 3 or a complimentary sequence;

d)   Nucleic acid molecules, the nucleic acid sequence of which has an identity of at least 50% with the nucleic acid sequences described under a) or c);

e)   Nucleic acid molecules, which hybridise with at least one strand of the nucleic acid molecules described under a) or c) under stringent conditions;

30 f)   Nucleic acid molecules, the nucleotide sequence of which deviates from the sequence of the nucleic acid molecules identified under a), b), c), d), e) or f) due to the degeneration of the genetic code; and

g)   Nucleic acid molecules, which represent fragments, allelic variants and/or derivatives of the nucleic acid molecules identified under a), b), c), d), e) or f).

In a further preferred embodiment of the method according to the invention, the foreign nucleic acid molecule is chosen from the group consisting of

a)   Nucleic acid molecules, which code at least one antisense RNA, which effects a reduction in the expression of at least one endogenous gene, which codes a Class 3 branching enzyme;

b)   Nucleic acid molecules, which by means of a co-suppression effect lead to the reduction in the expression of at least one endogenous gene, which codes a Class 3 branching enzyme;

c)   Nucleic acid molecules, which code at least one ribozyme, which splits specific transcripts of at least one endogenous gene, which codes a Class 3 branching enzyme;

d)   Nucleic acid molecules, which simultaneously code at least one antisense RNA and at least one sense RNA, wherein the said antisense RNA and the said sense RNA form a double-stranded RNA molecule, which effects a reduction in the expression of at least one endogenous gene, which codes a Class 3 branching enzyme (RNAi technology);

e)   Nucleic acid_molecules introduced by means of in vivo mutagenesis, which lead to a mutation or an insertion of a heterologous sequence in at least one endogenous gene coding a Class 3 branching enzyme, wherein the mutation or insertion effects a reduction in the expression of a gene coding a Class 3 branching enzyme or results in the synthesis of inactive Class 3 branching enzymes;

f)   Nucleic acid molecules, which code an antibody, wherein the antibody results in a reduction in the activity of a Class 3 branching enzyme due to the bonding to a Class 3 branching enzyme.

g)   Nucleic acid molecules, which contain transposons, wherein the integration of these transposons leads to a mutation or an insertion in at least one endogenous gene coding a Class 3 branching enzyme, which effects a reduction in the expression of at least one gene coding a Class 3 branching

enzyme, or results in the synthesis of inactive Class 3 branching enzymes; and/or

h) T-DNA molecules, which, due to insertion in at least one endogenous gene coding a Class 3 branching enzyme, effect a reduction in the expression of at

5   least one gene coding a Class 3 branching enzyme, or result in the synthesis of inactive Class 3 branching enzyme.

In a further embodiment of the method according to the invention, the genetically modified plants according to the invention synthesise a modified starch in

10 comparison with corresponding wild type plants that have not been genetically modified.

In a further embodiment of the method according to the invention, the method according to the invention is used to manufacture plants according to the invention.

15

The present invention also relates to the plants obtainable by the method according to the invention.

It is also an object of the present invention to provide means such as DNA

20 molecules, for example, for the production of plant cells according to the invention and plants according to the invention, which synthesise a modified starch in comparison with modified wild type plant cells or wild type plants that have not been genetically modified.

25 The present invention therefore also relates to nucleic acid molecules coding for a protein with the enzymatic activity of a Class 3 branching enzyme, chosen from the group consisting of

a) Nucleic acid molecules, which code a protein with the amino acid sequence given under Seq ID NO 4;

30 b) Nucleic acid molecules, which code a protein, which includes the amino acid sequence, which is coded by the insertion in plasmid DSM 15926;

c)   Nucleic acid molecules, which code a protein, the sequence of which has an identity of at least 70% with the amino acid sequence given under SEQ ID NO 4;

d)   Nucleic acid molecules, which code a protein, the sequence of which has an

5   identity of at least 70% with the amino acid sequence, which is coded by the insertion in plasmid DSM 15926;

e)   Nucleic acid molecules, which include the nucleotide sequence shown under Seq ID NO 3 or a complimentary sequence;

f)   Nucleic acid molecules, which include the nucleotide sequence of the insertion

10   contained in plasmid DSM 15926;

g)   Nucleic acid molecules, which have an identity of at least 70% with the nucleic acid sequences described under a), b), d) or e);

h)   Nucleic acid molecules, which hybridise with at least one strand of the nucleic acid molecules described under a), b), d), e) or f) under stringent conditions;

15   i)   Nucleic acid molecules, the nucleotide sequence of which deviates from the sequence of the nucleic acid molecules identified under a), b), e) or f) due to the degeneration of the genetic code; and

j)   Nucleic acid molecules, which represent fragments, allelic variants and/or derivatives of the nucleic acid molecules identified under a), b), c), d), e), f), g),

20   h) or i).

The amino acid sequence shown in SEQ ID NO 4 codes a protein with the activity of a Class 3 branching enzyme from *Solanum tuberosum*.

25   The proteins coded from the different varieties of nucleic acid molecules according to the invention have certain common characteristics. These can include, for example, biological activity, molecular weight, immunological reactivity, conformation etc, as well as physical characteristics such as, for example, the running behaviour in gel electrophoresis, chromatographic behaviour, sedimentation coefficients, solubility,

30   spectroscopic characteristics, stability; optimum pH, optimum temperature etc.
The molecular weight of the Class 3 branching enzyme from *Solanum tuberosum* derived from the amino acid sequence shown under SEQ ID NO 4 is ca. 103 kDa. The derived molecular weight of a protein according to the invention therefore

preferably lies in the range from 85 kDa to 120 kDa, preferably in the range from 95 kDa to 110 kDa and particularly preferably from ca. kDa 100 to 105 kDa.

5   The present invention relates to nucleic acid molecules, which code a protein with the enzymatic activity of a Class 3 branching enzyme, wherein the coded protein has an identity of at least 70%, preferably of at least 80%, particularly preferably of at least 90% and especially preferably of 95% with the amino acid sequence specified under SEQ ID NO 4.

10   A plasmid containing a cDNA, which codes a Class 3 branching enzyme from *Solanum tuberosum*, was deposited with the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, 38124 Braunschweig, Germany, in accordance with the Budapest Treaty on 15th September 2003 under the number DSM 15926. The amino acid sequence shown 15   SEQ ID NO 4 can be derived from the coding region of the cDNA sequence integrated in plasmid DSM 15926 and codes for a Class 3 branching enzyme from *Solanum tuberosum*. The present invention therefore also relates to nucleic acid molecules, which code a protein with the enzymatic activity of a Class 3 branching enzyme, which includes the amino acid sequence, which is coded by the insertion in 20   plasmid DSM 15926, wherein the coded protein has an identity of at least 70%, preferably of at least 80%, particularly preferably of at least 90% and especially preferably of 95% with the amino acid sequence, which can be derived from the insertion in DSM 15926.

25   The nucleic acid sequence shown SEQ ID NO 3 is a cDNA sequence, which includes the coding region for a Class 3 branching enzyme from *Solanum tuberosum*.
The present invention therefore also relates to nucleic acid molecules, which code a Class 3 branching enzyme and the coding region of the nucleotide sequence shown under Seq ID NO 3 or a complimentary sequence, nucleic acid molecules, which 30   include the coding region of the nucleotide sequence of the insertion contained in plasmid DSM 15926 and nucleic acid molecules, which have an identity of at least 70%, preferably of at least 80%, particularly preferably of at least 90% and especially preferably of at least 95% with the said nucleic acid molecules.

With the help of the sequence information of the nucleic acid molecule according to the invention or with the help of the nucleic acid molecule according to the invention, it is now possible for the person skilled in the art to isolate homologous sequences from other plant species, preferably from starch-storing plants, preferably from plant

5   species of the genus *Solanum*, particularly preferably from *Solanum tuberosum*. This can be carried out, for example, with the help of conventional methods such as the examination of cDNA or genomic banks with suitable hybridisation samples. The person skilled in the art knows that homologous sequences can also be isolated with the help of (degenerated) oligonucleotides and the use of PCR-based methods.

10   The examination of databases, such as are made available, for example, by EMBL (http://www.ebi.ac.uk/Tools/index.htm) or NCBI (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/), can also be used for identifying homologous sequences, which code for a Class 3 branching enzyme. In this case, one or more sequences are specified as a so-called query. This query sequence is

15   then compared by means of statistical computer programs with sequences, which are contained in the selected databases. Such database queries (e.g. blast or fasta searches) are known to the person skilled in the art and can be carried out by various providers.

If such a database query is carried out, e.g. at the NCBI (National Center for

20   Biotechnology Information, http://www.ncbi.nlm.nih.gov/), then the standard settings, which are specified for the particular comparison inquiry, should be used. For protein sequence comparisons (blastp), these are the following settings: Limit entrez = not activated; Filter = low complexity activated; Expect value = 10; word size = 3; Matrix = BLOSUM62; Gap costs: Existence = 11, Extension = 1.

25   For nucleic acid sequence comparisons (blastn), the following parameters must be set: Limit entrez = not activated; Filter = low complexity activated; Expect value = 10; word size = 11.

With such a database search, the sequences described in the present invention can be used as a query sequence in order to identify further nucleic acid molecules

30   and/or proteins, which code a Class 3 branching enzyme.

With the help of the described methods, it is also possible to identify and/or isolate nucleic acid molecules according to the invention, which hybridise with the sequence specified under SEQ ID NO 3 and which code a Class 3 branching enzyme.

Within the framework of the present invention, the term "hybridising" means hybridisation under conventional hybridisation conditions, preferably under stringent conditions such as, for example, are described in Sambrock et al., Molecular Cloning, A Laboratory Manual, 2nd Ed. (1989) Cold Spring Harbor Laboratory Press, Cold
5   Spring Harbor, NY). Particularly preferably, "hybridising" means hybridisation under the following conditions:

Hybridisation buffer:

2xSSC; 10xDenhardt solution (Ficoll 400+PEG+BSA; Ratio 1:1:1); 0.1% SDS; 5 mM EDTA; 50 mM Na_2HPO_4; 250 μg/ml herring sperm DNA; 50 μg/ml tRNA; or

10   25 M sodium phosphate buffer pH 7.2; 1 mM EDTA; 7% SDS

Hybridisation temperature: T=65 to 68°C

Wash buffer: 0.2xSSC; 0.1% SDS

Wash temperature: T=65 to 68°C.

In principle, nucleic acid molecules, which hybridise with the nucleic acid molecules
15   according to the invention, can originate from any plant species, which expresses an appropriate protein, preferably they originate from starch-storing plants, preferably from species of the genus Solanum, particularly preferably from Solanum tuberosum. Nucleic acid molecules, which hybridise with the molecules according to the invention, can, for example, be isolated from genomic or from cDNA libraries. The
20   identification and isolation of nuclear acid molecules of this type can be carried out using the nucleic acid molecules according to the invention or parts of these molecules or the reverse complements of these molecules, e.g. by means of hybridisation according to standard methods (see, for example, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2nd Ed. Cold Spring Harbor
25   Laboratory Press, Cold Spring Harbor, NY) or by amplification using PCR.

Nucleic acid molecules, which exactly or essentially have the nucleotide sequence specified under SEQ ID NO 3 or parts of this sequence, can be used as hybridisation samples. The fragments used as hybridisation samples can also be synthetic fragments or oligonucleotides, which have been manufactured using established
30   synthesising techniques and the sequence of which corresponds essentially with that of a nucleic acid molecule according to the invention. If genes have been identified and isolated, which hybridise with the nucleic acid sequences according to the invention, then a determination of this sequence and an analysis of the

characteristics of the proteins coded by this sequence should be carried out in order to establish whether a Class 3 branching enzyme is involved. Homology comparisons on the level of the nucleic acid or amino acid sequence and a determination of the enzymatic activity are particularly suitable for this purpose. As described above, the

5   activity of a Class 3 branching enzyme can take place by expression in E. coli strains, which themselves do not express an active branching enzyme (Kiel et al., 1987 Mol. Gen. Genet 207: 294-301); Guan et al., 1995, Proc. Natl. Acad. Sci. 92, 964-967).

The molecules hybridising with the nucleic acid molecules according to the invention

10   particularly include fragments, derivatives and allelic variants of the nucleic acid molecules according to the invention, which code a Class 3 branching enzyme from plants, preferably from starch-storing plants, preferably from plant species of the genus *Solanum*, particularly preferably from *Solanum tuberosum*. In conjunction with the present invention, the term "derivative" means that the sequences of these

15   molecules differ at one or more positions from the sequences of the nucleic acid molecules described above and have a high degree of identity with these sequences. Here, the deviation from the nucleic acid molecules described above can have come about, for example, due to deletion, addition, substitution, insertion or recombination.

20   Furthermore, identity means that functional and/or structural equivalence exists between the nucleic acid molecules concerned or the proteins coded by them. The nucleic acid molecules, which are homologous to the molecules described above and constitute derivatives of these molecules, are generally variations of these molecules, which constitute modifications, which execute the same biological function. At the

25   same time, the variations can occur naturally, for example they can be sequences from other plant species, or they can be mutations, wherein these mutations may have occurred in a natural manner or have been introduced by objective mutagenesis. The variations can also be synthetically manufactured sequences. The allelic variants can be both naturally occurring variants and also synthetically

30   manufactured variants or variants produced by recombinant DNA techniques. Nucleic acid molecules, which deviate from nucleic acid molecules according to the invention due to degeneration of the genetic code, constitute a special form of derivatives.

The proteins coded from the different derivatives of nucleic acid molecules according to the invention have certain common characteristics. These can include, for example, biological activity, substrate specificity, molecular weight, immunological reactivity, conformation etc, as well as physical characteristics such as, for example,

5   the running behaviour in gel electrophoresis, chromatographic behaviour, sedimentation coefficients, solubility, spectroscopic characteristics, stability; optimum pH, optimum temperature etc.

The nucleic acid molecules according to the invention can be any nucleic acid

10 molecules, in particular DNA or RNA molecules, for example cDNA, genomic DNA, mRNA etc. They can be naturally occurring molecules or molecules manufactured by genetic or chemical synthesis methods. They can be single-stranded molecules, which either contain the coding or the non-coding strand, or double-stranded molecules.

15

Furthermore, the present invention relates to nucleic acid molecules of at least 21, preferably more than 50 and particularly preferably more than 200 nucleotides length, which specifically hybridise with at least one nucleic acid molecule according to the invention. Here, specifically hybridise means that these molecules hybridise with

20 nucleic acid molecules, which code a protein according to the invention, but not with nucleic acid molecules, which code other proteins. In particular, the invention relates to such nucleic acid molecules, which hybridise with transcripts of nucleic acid molecules according to the invention and, as a result, can hinder their translation. Such nucleic acid molecules, which specifically hybridise with the nucleic acid

25 molecules according to the invention, can, for example, be constituents of antisense, RNAi or cosuppression constructs or ribozymes, or can be used as primers for PCR amplification.

In conjunction with the present invention, the term "identity" means a sequence

30 identity over the whole length of the coding region of at least 60%, in particular an identity of at least 70%, preferably greater than 80%, particularly preferably greater than 90% and especially of at least 95%. In conjunction with the present invention, the term "identity" is to be understood to mean the number of amino

acids/nucleotides (identity) corresponding with other proteins/nucleic acids, expressed as a percentage. Identity is preferably determined by comparing the Seq. ID NO 4 or SEQ ID NO 3 with other proteins/nucleic acids with the help of computer programs. If sequences that are compared with one another have different lengths,

5 the identity is to be determined in such a way that the number of amino acids, which have the shorter sequence in common with the longer sequence, determines the percentage quotient of the identity. Preferably, identity is determined by means of the computer program ClustalW, which is well known and available to the public (Thompson et al., Nucleic Acids Research 22 (1994), 4673-4680). ClustalW is made

10 publicly available by Julie Thompson (Thompson@EMBL-Heidelberg.DE) and Toby Gibson (Gibson@EMBL-Heidelberg.DE), European Molecular Biology Laboratory, Meyerhofstrasse 1, D 69117 Heidelberg, Germany. ClustalW can also be downloaded from different Internet sites, including the IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire, B.P.163, 67404 Illkirch Cedex, France;

15 ftp://ftp-igbmc.u-strasbg.fr/pub/) and the EBI (ftp://ftp.ebi.ac.uk/pub/software/) as well as from all mirrored Internet sites of the EBI (European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK).

Preferably, Version 1.8 of the ClustalW computer program is used to determine the identity between proteins according to the invention and other proteins. In doing so,

20 the following parameters must be set: KTUPLE=1, TOPDIAG=5, WINDOW=5, PAIRGAP=3, GAPOPEN=10, GAPEXTEND=0.05, GAPDIST=8, MAXDIV=40, MATRIX=GONNET, ENDGAPS(OFF), NOPGAP, NOHGAP.

Preferably, Version 1.8 of the ClustalW computer program is used to determine the identity between the nucleotide sequence of the nucleic acid molecules according to

25 the invention, for example, and the nucleotide sequence of other nucleic acid molecules. In doing so, the following parameters must be set:
KTUPLE=2, TOPDIAGS=4, PAIRGAP=5, DNAMATRIX:IUB, GAPOPEN=10, GAPEXT=5, MAXDIV=40, TRANSITIONS: unweighted.

30 Basically, nucleic acid molecules according to the invention, can originate from any plant, preferably they originate from starch-storing plants, preferably from plant species of the genus *Solanum*, particularly preferably from *Solanum tuberosum*.

Furthermore, the invention relates to vectors, in particular plasmids, cosmids, viruses, bacteriophages and other common vectors in genetic engineering, which contain the nucleic acid molecules according to the invention described above.

5   In a preferred embodiment, the nucleic acid molecules according to the invention contained in the vectors are linked with regulatory sequences, which guarantee expression in prokaryontic or eukaryontic cells. Here, the term "expression" can mean both transcription as well as transcription and translation. In this case, the nucleic acid molecules according to the invention can be present in "sense"
10 orientation and/or in "antisense" orientation to the regulatory sequences.

Regulatory sequences for expression in prokaryontic organisms, e.g. *E. coli*, and in eukaryontic organisms are adequately described in the literature, in particular those for expression in yeast such as *Saccharomyces cerevisiae*, for example. An overview
15 of different expression systems for proteins in different host organisms can be found, for example, in Methods in Enzymology 153 (1987), 383-516 and in Bitter et al. (Methods in Enzymology 153 (1987), 516-544).

For expressing the nucleic acid molecules, which code a Class 3 branching enzyme,
20 in sense and/or antisense orientation in vegetable cells, these are preferably linked with regulatory DNA sequences, which guarantee transcription in vegetable cells. In particular, these include promoters. In general, any promoter that is active in vegetable cells is eligible for expression.

At the same time, the promoter can be chosen so that expression takes place
25 constitutively or only in a certain tissue, at a certain stage of the plant development or at a time determined by external influences. The promoter can be homologous or heterologous both with respect to the plant and with respect to the nucleic acid molecule.

Suitable promoters are, for example, the promoter of the 35S RNA of the cauliflower
30 mosaic virus and the ubiquitin promoter from maize for constitutive expression, the patatin promoter B33 (Rocha-Sosa et al., EMBO J. 8 (1989), 23-29) for tuber-specific expression in potatoes or a promoter, which only ensures expression in photosynthetically active tissues, e.g. the ST-LS1 promoter (Stockhaus et al., Proc.

Natl. Acad. Sci. USA 84 (1987), 7943-7947; Stockhaus et al., EMBO J. 8 (1989), 2445-2451) or, for endosperm-specific expression of the HMG promoter from wheat, the USP promoter, the phaseolin promoter, promoters of zein genes from maize (Pedersen et al., Cell 29 (1982), 1015-1026; Quatroccio et al., Plant Mol. Biol. 15

5   (1990), 81-93), glutelin promoter (Leisy et al., Plant Mol. Biol. 14 (1990), 41-50; Zheng et al., Plant J. 4 (1993), 357-366; Yoshihara et al., FEBS Lett. 383 (1996), 213-218) or shrunken-1 promoter (Werr et al., EMBO J. 4 (1985), 1373-1380). However, promoters can also be used, which are only activated at a time determined by external influences (see for example WO 9307279). Promoters of heat-shock

10   proteins, which allow simple induction, can be of particular interest here. Furthermore, seed-specific promoters can be used, such as the USP promoter from *Vicia faba*, which guarantees seed-specific expression in *Vicia faba* and other plants (Fiedler et al., Plant Mol. Biol. 22 (1993), 669-679; Bäumlein et al., Mol. Gen. Genet. 225 (1991), 459-467).

15

Furthermore, a termination sequence (polyadenylation signal) can be present, which is used for adding a poly-A tail to the transcript. A function in the stabilisation of the transcripts is ascribed to the poly-A tail. Elements of this type are described in the literature (cf. Gielen et al., EMBO J. 8 (1989), 23-29) and can be exchanged at will.

20

In a further embodiment, the present invention relates to vectors, which contain DNA molecules, which code at least one antisense RNA, which effects a reduction in the expression of at least one endogenous gene, which codes a Class 3 branching enzyme.

25

In a further special embodiment, the present invention relates to vectors, which contain DNA molecules, which by means of a cosuppression effect lead to a reduction in the expression of at least one endogenous gene, which codes a Class 3 branching enzyme.

30

In a further embodiment, the present invention relates to vectors, which contain DNA molecules, which code at least one ribozyme, which splits specific transcripts of at least one endogenous gene, which codes a Class 3 branching enzyme.

In a further embodiment, the present invention relates to vectors, which contain DNA molecules, which simultaneously code at least one antisense RNA and at least one sense RNA, wherein the said antisense RNA and the said sense RNA form a double-
5   stranded RNA molecule, which effects a reduction in the expression of at least one endogenous gene, which codes a Class 3 branching enzyme (RNAi technology).

A further subject of the present invention is a host cell, in particular a prokaryontic or eukaryontic cell, which is genetically modified with a nucleic acid molecule according
10   to the invention and/or with a vector according to the invention, as well as cells, which originate from host cells of this type and which contain the genetic modification according to the invention.

In a preferred embodiment, the invention relates to host cells, in particular
15   prokaryontic or eukaryontic cells, which have been transformed using the nucleic acid molecule according to the invention or a vector according to the invention, as well as host cells, which originate from host cells of this type and which contain the described nucleic acid molecules or vectors according to the invention.
The host cells can be bacteria (e.g. *E. coli*) or fungus cells (e.g. yeast, in particular *S.*
20   *cerevisiae, Agaricus*, in particular *Agaricus bisporus*), as well as vegetable or animal cells. Here, the term "transforms" means that the cells according to the invention are genetically modified with a nucleic acid molecule according to the invention inasmuch as they contain at least one nucleic acid molecule according to the invention in addition to their natural genome. This can be freely present in the cell, possibly as a
25   self-replicating molecule, or it can be stably integrated in the genome of the host cell. The host cells are preferably microorganisms. Within the framework of the present application, these are understood to mean all bacteria and all protista (e.g. fungi, in particular yeast and algae), as defined, for example, in Schlegel "Allgemeine Mikrobiologie" (Georg Thieme Verlag (1985), 1-2).
30

It is especially preferred if the host cells according to the invention are plant cells. In principle, these can be plant cells from any plant species, i.e. both monocotyledonous and dicotyledonous plants. Preferably, these will be plant cells

from useful agricultural plants, i.e. from plants, which are cultivated by people for the purposes of food or for technical, in particular industrial purposes. The invention relates preferably to plant cells and plants from starch-storing plants (maize, rice, wheat, rye, oat, barley, cassava, potato, sago, mung bean, pea or sorghum); in

5   particular, plant cells from maize, rice, wheat or potato plants are particularly preferred.

A further subject of the present invention are proteins with the enzymatic activity of a Class 3 branching enzyme, chosen from the group consisting of

10   a)   Proteins, which include the amino acid sequence specified under SEQ ID NO 4;

    b)   Proteins, which are coded by the coding region of the DNA inserted in the plasmid DSM 15926; or

    c)   Proteins, which have an identity of at least 70% with the amino acid sequence
15   of the proteins identified under a) or b).

In a further embodiment, the present invention relates to proteins with the enzymatic activity of a Class 3 branching enzyme, wherein the coded protein has an identity of at least 70%, preferably of at least 80%, particularly preferably of at least 90% and

20   especially preferably of 95% with the amino acid sequence specified under SEQ ID NO 4 or with the amino acid sequence of a Class 3 branching enzyme coded by the insertion in plasmid DSM 15926.

In a further embodiment, the invention also relates to proteins, which are coded by

25   nucleic acid molecules according to the invention.

In a preferred embodiment, the present invention relates to a protein with the enzymatic activity of a Class 3 branching enzyme, wherein the Class 3 branching enzyme originates from a potato plant.

30

Surprisingly, it has been found that plant cells and plants, which have a reduced activity of a Class 3 branching enzyme, synthesise a starch, which is modified in comparison with starch from wild type plant cells or wild type plants.

In conjunction with the present invention, the term "modified starch" means that the starch has changed physical-chemical characteristics compared with non-modified starch obtainable from corresponding wild type plant cells or wild type plants that
5   have not been genetically modified.

In a preferred embodiment of the present invention, the modified starch is native starch.

In conjunction with the present invention, the term "native starch" means that the
10 starch is isolated from plants according to the invention, harvestable plant plants according to the invention or propagation material of plants according to the invention by methods known to the person skilled in the art.

Starch is a classical additive for many foodstuffs in which it essentially takes over the
15 function of binding aqueous additives or increasing the viscosity, or brings about an increased formation of gel. Important characteristic features are the flow and sorption behaviour, the source and sticking temperature, the viscosity and thickening performance, the solubility of the starch, the transparency and paste structure, the heat, shearing and acidic stability, the tendency to retrogradation, the ability to form a
20 film, the freezing/thawing stability, the digestibility as well as the ability to form complexes with, for example, inorganic or organic ions.

In the area of the non-foodstuffs industry, starch can be used, for example, as an auxiliary substance for different manufacturing processes or as an additive in technical products. Particular mention must be made here of the paper and
25 cardboard industry where starch is used as an auxiliary substance. Here, the starch is primarily used for retardation (holding back of solids), the bonding of filler and fine material particles, as a consolidation material and for dehydration. In addition to this, the favourable characteristics of starch with regard to stiffness, hardness, sound, grip, shine, smoothness and resistance to splitting as well as the surfaces are also
30 fully utilised.

A further major area of use of starches is in the adhesive industry, where the possible applications are divided into four sub-areas. Use as a pure starch adhesive, use with starch adhesives prepared with special chemicals, use of starch as an additive to

synthetic resins and polymer dispersions, and the use of starches as a stretching medium for synthetic adhesives.

Furthermore, starches can be used as additives for building materials (e.g. plasterboard sheets, ready-mixed concrete, plaster and mineral fibres), for the manufacture of media for stabilising soil, as a functional aid in plant protection media or fertilisers, as a functional aid in the pharmaceutical industry (e.g. as a bonding medium, tablet dispersal medium, in lubricating and vulnerary powders) and the cosmetic industry (as a carrier of additives), as a strengthening additive for coal and briquettes, as a flocculation medium (e.g. in the preparation of carbon sludge) and as a bonding medium, e.g. in Betonit.

Plant cells according to the invention and plants according to the invention synthesise a modified starch in comparison with starch of corresponding wild type plant cells or wild type plants that have not been genetically modified. In its physical-chemical characteristics, e.g. the amylopectin/amylose ratio, the degree of branching, the phosphate content, the average chain length, the viscosity behaviour, the starch grain size, the side chain distribution and/or the starch grain form, the modified starch is changed in comparison with the synthesised starch in wild type plant cells or plants so that it is better suited for use in particular application areas, for example.

The present invention therefore also relates to modified starches obtainable or isolated from plant cells according to the invention or plants according to the invention, from propagation material according to the invention or from harvestable plant parts according to the invention.

In a particularly preferred embodiment, the present invention relates to modified potato starch.

Furthermore the present invention relates to a method for the manufacture of a modified starch including the step of extracting the starch from a plant cell according to the invention or from a plant according to the invention, from propagation material according to the invention of such a plant and/or from harvestable plant parts according to the invention of such a plant, preferably from starch-storing parts

according to the invention of a plant. Preferably, such a method also includes the step of harvesting the cultivated plants or plant parts and/or the propagation material of these plants before the extraction of the starch and, further, particularly preferably the step of cultivating plants according to the invention before harvesting.

5

Methods for extracting starches from plants or from starch-storing parts of plants are known to the person skilled in the art. Furthermore, methods for extracting starch from different starch-storing plants are described, e.g. in Starch: Chemistry and Technology (Publisher: Whistler, BeMiller and Paschall (1994), 2nd Edition,

10 Academic Press Inc. London Ltd; ISBN 0-12-746270-8; see e.g. Chapter XII, Page 412-468: Maize and Sorghum Starches: Manufacture; by Watson; Chapter XIII, Page 469-479: Tapioca, Arrowroot and Sago Starches: Manufacture; by Corbishley and Miller; Chapter XIV, Page 479-490: Potato starch: Manufacture and Uses; by Mitch; Chapter XV, Page 491 to 506: Wheat starch: Manufacture, Modification and Uses; by

15 Knight and Oson; and Chapter XVI, Page 507 to 528: Rice starch: Manufacture and Uses; by Rohmer and Klem; Maize starch: Eckhoff et al., Cereal Chem. 73 (1996), 54-57, the extraction of maize starch on an industrial scale is generally achieved by so-called "wet milling".). Devices, which are in common use in methods for extracting starch from plant material are separators, decanters, hydrocyclones, spray dryers

20 and fluid bed dryers.

In conjunction with the present invention, the term "starch-storing parts" is to be understood to mean such parts of a plant in which, in contrast to transitory leaf starch, starch is stored as a deposit for surviving for longer periods. Preferred starch-

25 storing parts are tubers, storage roots, seeds or endosperm; particularly preferred are potato tubers or the endosperm of maize, wheat or rice plants.

Modified starch obtainable by the method according to the invention is also the subject matter of the present invention.

30

Furthermore, the use of plant cells according to the invention or plants according to the invention for manufacturing a modified starch are the subject matter of the present invention.

The person skilled in the art knows that the characteristics of starch can be changed by thermal, chemical, enzymatic or mechanical derivation, for example. Derived starches are particularly suitable for different applications in the foodstuffs and/or

5 non-foodstuffs sector. The starches according to the invention are better suited as a starting substance for the manufacture of derived starches than conventional starches. In the manufacture of derived starch, they are distinguished by better processing capability and lead to new products, as a modified starch is used as a new starting material for the derivation process.

10

The present invention therefore also relates to the manufacture of a derived starch, wherein modified starch according to the invention is derived retrospectively.

In conjunction with the present invention, the term "derived starch" is to be

15 understood to mean a modified starch according to the invention, the characteristics of which have been retrospectively changed after isolation from vegetable cells with the help of chemical, enzymatic, thermal or mechanical methods.

In a preferred embodiment of the present invention, the derived starch according to the invention is starch that has been heat-treated and/or acid-treated.

20 In a further preferred embodiment, the derived starches are starch ethers, in particular starch alkyl ethers, O-allyl ethers, hydroxylalkyl ethers, O-carboxylmethyl ethers, nitrogen-containing starch ethers, phosphate-containing starch ethers or sulphur-containing starch ethers.

25 In a further preferred embodiment, the derived starches are cross-linked starches.

In a further preferred embodiment, the derived starches are starch graft polymers.

In a further preferred embodiment, the derived starches are oxidised starches.

30

In a further preferred embodiment, the derived starches are starch esters, in particular starch esters, which have been introduced into the starch using organic

acids. Particularly preferably these are phosphate, nitrate, sulphate, xanthate, acetate or citrate starches.

5   The derived starches according to the invention are suitable for different applications in the foodstuffs and/or non-foodstuffs sector. Methods for manufacturing derived starches according to the invention are known to the person skilled in the art and are adequately described in the general literature. An overview on the manufacture of derived starches can be found, for example, in Orthoefer (in Corn, Chemistry and Technology, 1987, eds. Watson und Ramstad, Chapter 16, 479-499).

10

Derived starch obtainable by the method according to the invention for manufacturing a derived starch is also the subject matter of the present invention.

Furthermore, the use of modified starches according to the invention for
15   manufacturing derived starch is the subject matter of the present invention.

Description of sequences

SEQ ID NO 1:   Nucleic acid sequence containing the coding region of the 3'-
20   area of a Class 3 branching enzyme from *Solanum tuberosum* (cv Désirée). This sequence is inserted in plasmid AN 46-196.

SEQ ID NO 2:   Nucleic acid sequence containing the coding region of the 5'-
area of a Class 3 branching enzyme from *Solanum tuberosum* (cv Désirée). This
25   sequence is inserted in plasmid AN 47-196.

SEQ ID NO 3:   Nucleic acid sequence containing the full coding region of a Class 3 branching enzyme from *Solanum tuberosum* (cv Désirée). This sequence is inserted in plasmid AN 49 and was deposited with the Deutsche Sammlung von
30   Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, 38124 Braunschweig, Germany, in accordance with the Budapest Treaty on 15th September 2003 under the number DSM 15926.

SEQ ID NO 4:   Amino acid sequence coding a Class 3 branching enzyme from *Solanum tuberosum* (cv Désirée). This sequence can be derived from the nucleic acid sequence inserted in plasmid AN 49 or from the nucleic acid sequence described under SEQ ID NO 3.

5

SEQ ID NO 5:   Nucleic acid sequence containing the full coding region of a Class 3 branching enzyme from *Solanum tuberosum* (cv Désirée). This sequence has been obtained by combining the nucleic acid sequences described under SEQ ID NO 1 and SEQ ID NO 2. This nucleic acid sequence constitutes an allelic variant

10   of the nucleic acid sequence described under SEQ ID NO 3 coding a Class 3 branching enzyme.

SEQ ID NO 6:   Amino acid sequence coding a Class 3 branching enzyme from *Solanum tuberosum* (cv Désirée). This sequence can be derived from the nucleic

15   acid sequence described under SEQ ID NO 5 and constitutes an allelic variant of the amino acid sequence described under SEQ ID NO 4 coding a Class 3 branching enzyme

General methods

20   The following methods were used in the examples:

Demonstration of the activity of a Class 3 branching enzyme

The activity of a Class 3 branching enzyme was demonstrated with the help of non-denaturing gel electrophoresis as follows:

25   To isolate proteins from plants, the test material was ground with a pestle in liquid nitrogen, absorbed into an extraction buffer (50 mM Na citrate, pH 6.5; 1 mM EDTA, 4 mM DTT) and, after centrifugation (10 min, 14.000 g, 4 °C), was used directly for measurement of the protein content according to Bradford. Subsequently, 5µg to 20 µg total protein extract was mixed with 4X loading buffer (20% glycerol, 125 mM Tris

30   HCl, pH 6.8) and loaded onto a BE activity gel. The BE activity gel was made up as follows: 2.5 ml 30% acrylamide:0.8% bisacrylamide, 0.1 ml running buffer, 7.4 ml H_2O, 10% ammonium persulphate solution and 5 µl N,N,N',N'-

tetramethylethylenediamine (TEMED). The running buffer (RB) was made up as follows: RB = 30.2 g Tris base, pH 8.0, 144 g glycine on 1 L H_2O. On completion of the gel run, each of the gels was incubated overnight at 37 °C in 25 ml "phosphorylase buffer" (25 ml 1M Na citrate pH 7.0, 0.47 g glucose-1-phosphate,

5   12.5 mg AMP, 2.5 mg phosphorylase a/b from "rabbit"). The gels were coloured with Lugol's solution.

Starch analysis

a)   Determination of the amylose content and of the amylose/amylopectin ratio

10   Starch was isolated from potato plants by standard methods, and the amylose content and the amylose:amylopectin ratio was determined by the method described by Hovenkamp-Hermelink et al. (Potato Research 31, (1988), 241-246).

b)   Determination of the phosphate content

15   In starch, the positions C2, C3 and C6 of the glucose units can be phosphorylated. To determine the C6-P content of starch, 50 mg of starch are hydrolysed for 4 h at 95°C in 500 µl of 0.7 M HCl. The samples are then centrifuged for 10 minutes at 15500xg and the supernatants are removed. 7 µl of the supernatants are mixed with 193 µl of imidazole buffer (100 mM imidazole, pH 7.4; 5 mM $MgCl_2$, 1 mM EDTA and

20   0.4 mM NAD). The measurement was carried out in a photometer at 340 nm. After the base absorption had been established, the enzyme reaction was started by addition of 2 units glucose-6-phosphate dehydrogenase (from Leuconostoc mesenteroides, Boehringer Mannheim). The change in absorption is directly proportional to the concentration of the G-6-P content of the starch.

25

The total phosphate content was determined by the method of Ames (Methods in Enzymology VIII, (1966), 115-118).

Approximately 50 mg of starch are treated with 30 µl of ethanolic magnesium nitrate solution and ashed for 3 hours at 500°C in a muffle oven. The residue is treated with

30   300 µl of 0.5 M hydrochloric acid and incubated for 30 minutes at 60°C. One aliquot is subsequently made up to 300 µl 0.5 M hydrochloric acid and this is added to a

mixture of 100 µl of 10% ascorbic acid and 600 µl of 0.42% ammonium molybdate in 2 M sulphuric acid and incubated for 20 minutes at 45°C.

This is followed by a photometric determination at 820 nm with a phosphate calibration series as standard.

5

c)   Determination of the viscosity characteristics by means of a Rapid Visco Analyser (RVA)

2 g of starch (DM) are taken up in 25 ml of H_2O (VE-type water, conductivity of at
10   least 15 mega ohm) and used for the analysis in a Rapid Visco Analyser Super3 (Newport Scientific Pty Ltd., Investmet Support Group, Warriewod NSW 2102, Australia). The apparatus is operated following the manufacturer's instructions. The viscosity values are indicated in Centipoise (cP) in accordance with the manufacturer's operating manual, which is incorporated into the description herewith
15   by reference. To determine the viscosity of the aqueous starch solution, the starch suspension is first stirred for 10 seconds at 960 rpm and subsequently heated at 50°C at a stirring speed of 160 rpm, initially for a minute (step 1). The temperature was then raised from 50°C to 95°C at a heating rate of 12°C per minute (step 2). The temperature is held for 2.5 minutes at 95°C (step 3) and then cooled from 95°C to
20   50°C at 12°C per minute (step 4). In the last step (step 5), the temperature of 50°C is held for 2 minutes. The viscosity is determined during the entire duration.

After the programme has ended, the stirrer is removed and the beaker covered. The gelatinized starch is now available for the texture analysis after 24 hours incubation at room temperature.

25

The profile of the RVA analysis contains parameters which are shown for the comparison of different measurements and substances. In the context of the present invention, the following terms are to be understood as follows:

1.   Maximum viscosity (RVA Max)
30   The maximum viscosity is understood as meaning the highest viscosity value, measured in cP, obtained in step 2 or 3 of the temperature profile.

2.   Minimum viscosity (RVA Min)

The minimum viscosity is understood as meaning the lowest viscosity value, measured in cP, observed in the temperature profile after the maximum viscosity. Normally, this takes place in step 3 of the temperature profile.

3.   Final viscosity (RVA Fin)

5   The final viscosity is understood as meaning the viscosity value, measured in cP, observed at the end of the measurement.

4.   Setback (RVA Set)

What is known as the "setback" is calculated by subtracting the value of the final viscosity from that of the minimum occurring after the maximum viscosity in the

10   curve.

5.   Gelatinization temperature (RVA PT)

The gelatinization temperature is understood as meaning the point in time of the temperature profile where, for the first time, the viscosity increases drastically for a brief period.

15

d)   Determination of the gel strength (Texture Analyser)

2 g of starch (DM) are gelatinized in the RVA apparatus in 25 ml of an aqueous suspension (temperature programme: see item d) "Determination of the viscosity characteristics by means of a Rapid Visco Analyser (RVA)") and subsequently stored

20   for 24 hours at room temperature in a sealed container. The samples are fixed under the probe (round piston with planar surface) of a Texture Analyser TA-XT2 from Stable Micro Systems (Surrey, UK) and the gel strength was determined using the following parameters:

-	Test speed	0.5 mm/s
-	Depth of penetration	7 mm
-	Contact surface	113 mm^2
-	Pressure	2 g

e)   Analysis of the side-chain distribution of the amylopectin by means of ion-
30   exchange chromatography

To separate amylose and amylopectin, 200 mg of starch are dissolved in 50 ml reaction vessels, using 12 ml of 90% (v/v) DMSO in H_2O. After addition of 3 volumes of ethanol, the precipitate is separated by centrifugation for 10 minutes at about

1800xg at room temperature (RT). The pellet is then washed with 30 ml of ethanol, dried and dissolved in 40 ml of 1% (w/v) NaCl solution at 75°C. After the solution has cooled to 30°C, approximately 90 mg of thymol are added slowly, and this solution is incubated for at least 60 h at 30°C. The solution is then centrifuged for 30 minutes at

5   2000xg (RT). The supernatant is then treated with 3 volumes of ethanol, and the amylopectin which settles out is separated by centrifugation for 5 minutes at 2000xg (RT). The pellet (amylopectin) is then washed with ethanol and dried using acetone. By addition of DMSO to the pellet, one obtains a 1% solution, of which 200 µl are treated with 345 µl of water, 10 µl of 0.5 M sodium acetate (pH 3.5) and 5 µl of

10   isoamylase (dilution 1:10; Megazyme) and incubated for about 16 hours at 37°C. A 1:5 aqueous dilution of this digest is subsequently filtered through a 0.2 µm filter, and 100 µl of the filtrate are analysed by ion chromatography (HPAEC-PAD, Dionex). Separation was performed using a PA-100 column (with suitable precolumn), while detection was performed amperometrically. The elution conditions were as follows:

15   Solution A - 0.15M NaOH

Solution B – 1 M sodium acetate in 0.15M NaOH

t (min)	Solution A (%)	Solution B (%)
5	0	100
35	30	70
45	32	68
60	100	0
70	100	0
72	0	100
80	0	100
Stop		

Table 1: Composition of the elution buffer for the side chain analysis of the
20   amylopectin at different times during the HPEAC-PAD Dionex analysis. Between the times stated, the composition of the elution buffer changes in each case linearly.

The determination of the relative amount of short side chains in the total of all side chains is carried out via the determination of the percentage of a particular side chain

in the total of all side chains. The total of all side chains is determined via the determination of the total area under the peaks which represent the polymerization degrees of DP 6 to 34 in the HPCL chromatogram.

The percentage of a particular side chain in the total of all side chains is determined

5   via the determination of the ratio of the area under the peak which represents this side chain in the HPLC chromatogram to the total area. The programme Chromelion 6.20 Version 6.20 from Dionex, USA, was used for determining the peak areas.

f)   Determination of the activity of the BEIII protein

10   This was carried out as specified in the example.

g)   DSC-analysis („Differential Scanning Calorimetry")

Investigations with the aid of DSC-analysis have been done by the method described by WO 01/19975. 10 mg starch treated with 30 µl H_2O (VE-type water, conductivity of

15   at least 15 mega ohm) were sealed in stainless steal pans (volume 50 µl). The pan is heated from 20°C to 150°C at a rate of 10°C per minute in a Diamond DSC-instrument (Perkin Elmer). The programme Pyres from Perkin Elmer was used for determining the data.

20

Examples

Example 1

Cloning of a full-length sequence coding a Class 3 branching enzyme

25   **from Solanum tuberosum**

The gene sequence coding for this Class 3 branching enzyme in Solanum tuberosum has not previously been described .

By sequence comparisons with different branching enzymes, a domain was identified, with the help of which EST databases were examined. In doing so, the

30   EST   TC73137   (TIGR   database;   http://www.tigr.org/tigr-scripts/tgi/tc_report.pl?tc=TC73137&species=potato) from potato was identified.

With the help of the primers B1_Asp (GAT GGG TAC CAG CAC TTC TAC TTG GCA GAG G) and B2_Sal (TCA AGT CGA CCA CAA CCA GTC CAT TTC TGG), a sequence from a tuber-specific cDNA bank from *Solanum tuberosum* (cv. Désirée) corresponding to this EST sequence was amplified. Attempts to use leaf-specific,

5   "sink"-tissue-specific or "source"-tissue-specific cDNA banks as a template for the PCR reaction led to no amplification.

In order to amplify the whole coding sequence of the branching enzyme concerned, which up to now had also included unknown sequences, primers were manufactured, which were complimentary to the ends of the previously known sequence and vector

10   sequences of the cDNA banks concerned. With all the primer combinations for the amplification of a full-length sequence of a Class 3 branching enzyme used in this approach, it was not possible to amplify any further area. Hereupon, EST databases of tomato were examined again.

In this case, two ESTs from tomato were identified (TIGR database; BG127920 and

15   TC130382), which either had a high homology to the amplification of the Class 3 branching enzyme from potato described above (TC130382) and (BG127920) respectively, or to the putative branching enzyme gene from arabidopsis (GenBank: GP|9294564|dbj|BAB02827.1).

20   Primers were now manufactured again in order to also amplify previously unknown sequences of the Class 3 branching enzyme. By means of PCR, the 3'-area of the Class 3 branching enzyme was amplified from a cDNA bank, made from tubers of *Solanum tuberosum* (cv. Désirée), with the primers KM2_Spe (5'-TCAAACTAGTCACAACCAGTCCATTTCTGG-3') and So_putE (5'-

25   CACTTTAGAAGGTATCAGAGC-3'). The fragment with a size of ca. 1 kb that was obtained was cloned undirectedly in the pCR4-TOPO vector from Invitrogen (product number: 45-0030). The plasmid produced was designated as AN 46-196. The sequence of the inserted fragments in the plasmid AN 46-196 is shown under SEQ ID NO 1.

30

The 5'-area was likewise amplified by means of PCR technology and using the primers So_put5' (5'-GTATTTCTGCGAAGGAACGACC-3') and So_putA (5'-AACAATGCTCTCTCTGTCGG-3') from the same cDNA bank. The fragment with a

size of ca. 2 kb that was obtained was cloned undirectedly in the pCR4-TOPO vector from Invitrogen (product number: 45-0030). The plasmid produced was designated as AN 47-196. The sequence of the inserted fragments in the plasmid AN 47-196 is shown under SEQ ID NO 2.

5

Primers were now manufactured again in order to amplify a full-length sequence. The following primers were used: SO_putA (AACAATGCTCTCTCTGTCGG) and SO_putE (CACTTTAGAAGGTATCAGAGC). A PCR product with an approximate size of 3.2 kb was obtained and was cloned in the pCR2.1 vector from Invitrogen 10   (product number: 45-0030). The plasmid obtained (filed under DSM 15926) was designated as AN 49. The sequence of the inserted fragments in the plasmid AN 49 is shown under SEQ ID NO 3.

Example 2

15   **Information on vectors and plasmids**

Information on vector AN 54-196

AN 54-196 is a derivative of the plasmid pBinB33-Hyg, to which was added a part sequence of the Class 3 branching enzyme gene as an "inverted repeat, (RNAi technology) under the control of the promoters of the patatin gene B33 from *Solanum* 20   *tuberosum* (Rocha-Sosa et al., 1989). For this purpose, first of all, a PCR product with the primers B1_Asp (GAT GGG TAC CAG CAC TTC TAC TTG GCA GAG G) and B2_Sal (TCA AGT CGA CCA CAA CCA GTC CAT TTC TGG) from a tuber-specific cDNA bank from *Solanum tuberosum* (cv. Désirée) was amplified, as a result of which the sites Asp718 and SalI were added. The PCR product obtained (625 bp) 25   was cloned in "antisense" orientation to the B33 promoter via these two sites. A second PCR fragment, which was amplified with the primers B3_Sal (GCT TGT CGA CGG GAG AAT TTT GTC CAG AGG) and B4_Sal (GAT CGT CGA CAG CAC TTC TAC TTG GCA GAG G) from a tuber-specific cDNA bank from *Solanum tuberosum* (cv. Désirée) and which is identical to the 301 bp of the first fragment, was cloned via

the SalI site behind the first fragment, but in "sense" orientation to the B33 promoter. This arrangement is described as "inverted repeat" (RNAi technology).

Information on vector pBinB33-Hyg

5   Starting from the plasmid pBinB33, the EcoRI-HindIII fragment including the B33 promoter, a part of the polylinker, and the ocs terminator were cut out and spliced into the correspondingly cut vector pBIB-Hyg (Becker, 1990).

The plasmid pBinB33 was obtained by splicing the promoter of the patatin gene B33 from Solanum tuberosum (Rocha-Sosa et al., 1989) as a DraI fragment (nucleotide –

10 1512 - +14) into the vector pUC19 cut with SstI, the ends of which had been smoothed with the help of the T4 DNA polymerase. This resulted in the plasmid pUC19-B33. The B33 promoter was cut out from this plasmid with EcoRI and SmaI and spliced into the correspondingly cut vector pBinAR. This resulted in the vegetable expression vector pBinB33.

15 The plasmid pBinAR is a derivative of the vector plasmid pBin19 (Bevan, 1984) and was constructed as follows:

A fragment of length 529 Bp, which included the nucleotides 6909-7437 of the 35S RNA promoter of the cauliflower mosaic virus (Pietrzak et al., 1986, Nucleic Acids Research 14, 5857-5868), was isolated as an EcoRI/KpnI fragment from the plasmid

20 pDH51 (Pietrzak et al., 1986) and spliced between the EcoRI and KpnI sites of the polylinker from pUC18. This resulted in the plasmid pUC18-35S.

With the help of the restriction endonucleases HindIII und PvuII, a fragment of length 192 Bp, which included the polyadenylation signal (3'-end) of the octopin synthase gene (gene 3) of the T-DNA of the Ti plasmid pTiACH5 (Gielen et al., 1984)

25 (nucleotides 11749-11939) was isolated from the plasmid pAGV40 (Herrera-Estrella et al., 1983). After the addition of SspI linkers to the PvuII site, the fragment was spliced between the SphI and HindIII site from pUC18-35S. This resulted in the plasmid pA7.

The whole polylinker containing the 35S promoter and the ocs terminator with EcoRI

30 and HindIII was cut out of pA7 and spliced into the correspondingly cut pBin19. This resulted in the vegetable expression vector pBinAR (Höfgen and Willmitzer, 1990).

Example 3

Genetically modified plants with reduced Class 3 branching enzyme activity

5   In order to produce transgenic potato plants, which have a reduced expression of a Class 3 branching enzyme gene, the T-DNA of the plasmid AN 54-196 was transferred into potato plants of the variety Désirée with the help of agrobacteria, as described in Rocha-Sosa et al. (EMBO J. 8, (1989), 23-29). The plants of the variety Désirée obtained by transformation with the plasmid AN 53-196 were designated as

10   369SO.

Analysis with the help of non-denaturising gel electrophoresis of protein extracts from tubers of wild type plant cells and/or protein extracts from genetically modified plants (396SO), showed that the genetically modified plant cells have a reduced activity of a Class 3 branching enzyme in comparison with protein extracts from tubers of wild

15   type plant cells.

Additionally mRNA of tuber material was extracted with standard methods and applied to quantitative RT-PCR analysis. The analysis were performed with a PCR-instrument ABI Prism 7700 form Applied Biosystems using the primer St_BE-f2 (5'-TCA GGT CTA CAA GTT GAC CCG A-3'), St_BE-r2 (5'-GTA GAA CCT TCC CTT

20   TTG TGT GA-3') and St_BE-Fam (5'-Fam-CAT GAT CAC TCT AGC AAT CAA AGT GCC-Tamra-3'). It could be shown that given plants showed reduced transcript in comparison with the corresponding wild type.

Example 4

25   **Potato starch extraction process**

All tubers of one line (0,3 to 0,7 kg) are processed jointly in a commercially available juice extractor (Multipress automatic MP80, Braun). The starch-containing fruit water is collected in a 1-l bucket (ratio bucket height: bucket diameter = approx. 1.1) containing 20 ml of tap water together with a spoon-tipful (approx. 0,3-0,4 g) of

sodium disulphite. The bucket is subsequently filled completely with tap water. After the starch has been allowed to settle for 2 hours (h), the supernatant is decanted off, the starch is resuspended in 1 l of tap water and poured over a sieve with a mesh size of 125 μm. After 2 h (starch has again settled at the bottom of the bucket), the
5 aqueous supernatant is again decanted off. This wash step is repeated 3 more times so that the starch is resuspended a total of 5 times in fresh tap water. Thereafter, the starches are dried at 37°C to a water content of 12-17% and homogenized using a pestle and mortar. The starches are now available for analyses.

10 *Example 5*

Analysis of the starch from plants with reduced BEIII gene expression

The starch from various independent lines of plants named 369SO were isolated from potato tubers. The physico-chemical properties of this starch were subsequently analysed. The results of the characterization of the modified starches are shown in
15 the following for an example of a selection of certain plant lines. The analyses were carried out by the methods described hereinabove.

a)   RVA Analysis

	RVA Max (%)	RVA Min (%)	RVA Fin (%)	RVA Set (%)	RVA PT (%)	Gel strength
cv.Desiree	100	100	100	100	100	100
369SO048	91	64	90	N.d.	98	128
369SO050	84	84	89	112	98	127
369SO052	94	85	88	101	98	N.d.
369SO106	91	87	89	99	98	N.d.
369SO129	87	88	93	114	99	138

Table 2: Parameters of the RVA analysis of starch isolated from wild-type plants (cv.
20 Desiree), plants with a reduced activity of a BEIII protein (369SO) in per cent based on data of starch of the wild type. The RVA analysis was carried out as described in general methods. N.d. = not determined.

66

b)　　Analysis of the phosphate and Amylose content

No.	Genotype	Phosphate in C6 (%)	Total phosphate in (%)	Amylose (%)	Amylose (% WT)
1	cv. Desiree	100,0	100,0	21,3	100,0
2	369SO048	72,8	84,8	20,8	97,7
3	369SO050	79,2	78,0	20,1	94,4
4	369SO052	84,8	83,2	19,6	92,0
5	369SO106	84,8	85,9	20,1	94,4
6	369SO129	80,8	81,2	20,2	94,8

Table 3: Phosphate and amylose contents of starch isolated from wild-type plants (cv. Desiree), plants with a reduced activity of BEIII protein (369SO). The phosphate contents in the C6 position of the glucose monomers and the total phosphate content of the starch are indicated in per cent based on starch from wild-type plants; amylose contents are indicated in per cent amylose based on the total amount of the starch, or in per cent based on the amylose content of starch from wild-type plants.

c)　　Analysis of side-chain distribution

The analysis of the side-chain distribution of the amylopectin was carried out as described above. The table which follows is a summary of the contributions of the individual peak areas:

Glucose units	cv. Desiree	369SO 048	369SO 050	369SO 052	369SO 106	369SO 129
dp 6	2,19	2,57	2,83	2,78	2,59	2,59
dp 7	1,69	1,76	1,84	1,85	1,85	1,73
dp 8	1,35	1,34	1,37	1,38	1,44	1,36
dp 9	2,26	2,27	2,31	2,32	2,42	2,31
dp 10	3,74	3,81	3,86	3,94	4,00	3,85
dp 11	5,13	5,23	5,30	5,45	5,37	5,30
dp 12	5,99	6,14	6,18	6,32	6,17	6,17
dp 13	6,40	6,53	6,54	6,63	6,48	6,63
dp 14	6,39	6,45	6,44	6,49	6,37	6,52
dp 15	6,11	6,14	6,12	6,15	6,05	6,09
dp 16	5,74	5,75	5,72	5,75	5,68	5,72
dp 17	5,37	5,35	5,35	5,35	5,30	5,35
dp 18	5,08	5,04	5,06	5,05	5,01	5,06
dp 19	4,89	4,86	4,88	4,84	4,83	4,86
dp 20	4,68	4,59	4,65	4,60	4,60	4,62

Glucose units	cv. Desiree	369SO 048	369SO 050	369SO 052	369SO 106	369SO 129
dp 6	100	117,4	129,2	126,9	118,3	118,3
dp 7	100	104,1	108,9	109,5	109,5	102,4
dp 8	100	99,3	101,5	102,2	106,7	100,7
dp 9	100	100,7	102,4	102,9	107,3	102,4
dp 10	100	102,0	103,3	105,5	107,1	103,1
dp 11	100	102,0	103,4	106,3	104,8	103,4
dp 12	100	102,5	103,2	105,5	103,0	103,0
dp 13	100	102,1	102,3	103,7	101,3	103,7
dp 14	100	100,9	100,8	101,6	99,7	102,0
dp 15	100	100,5	100,2	100,7	99,0	99,7
dp 16	100	100,3	99,7	100,3	99,0	99,7
dp 17	100	99,7	99,7	99,7	98,8	99,7
dp 18	100	99,3	99,7	99,5	98,7	99,7
dp 19	100	99,5	99,9	99,1	98,9	99,5
dp 20	100	98,1	99,4	98,3	98,3	98,7
dp 21	100	99,5	99,1	98,0	98,4	98,6
dp 22	100	98,8	99,0	97,6	98,0	98,0
dp 23	100	98,8	97,7	96,2	98,3	98,8
dp 24	100	98,4	96,9	96,0	98,1	96,9
dp 25	100	96,9	95,9	94,0	96,6	97,2
dp 26	100	97,1	94,2	93,5	96,4	95,6
dp 27	100	95,8	93,8	91,7	95,0	95,0
dp 28	100	96,1	92,3	91,3	95,2	93,7
dp 29	100	97,2	92,0	90,3	95,5	94,9
dp 30	100	94,0	91,4	89,4	93,4	93,4
dp 31	100	95,6	90,8	89,2	92,4	94,0
dp 32	100	94,2	89,3	89,3	92,2	92,2
dp 33	100	92,2	89,8	91,0	93,4	93,4
dp 34	100	91,9	88,9	90,4	93,3	91,9

Table 5: The table shows a summary of the contributions of the individual peak areas of the HPAEC chromatogram in per cent based on starch from wild-type plants.

d)   Analysis of the amylopectin side chain distribution by means of gel permeation
      chromatography

Analysis of the amylopectin side chain distribution by means of gel permeation chromatography were additionally performed.

To separate amylose and amylopectin, 100 mg of starch are dissolved in 6 ml of 90% strength (v/v) DMSO with constant stirring. After addition of 3 volumes of ethanol, the precipitate is separated off by centrifugation for 10 minutes at 1800xg at room temperature. The pellet is subsequently washed with 30 ml of ethanol, dried and dissolved in 10 ml of 1% strength (w/v) NaCl solution at 60°C. After cooling the

solution to 30°C, approximately 50 mg of thymol are added slowly, and this solution is incubated for 2 to 3 days at 30°C. The solution is subsequently centrifuged for 30 minutes at 2000xg at room temperature. The supernatant is treated with three volumes of ethanol, and the amylopectin which precipitates is separated off by

5   centrifugation for 5 minutes at 2000xg at room temperature. The pellet (amylopectin) is washed with 10 ml of 70% strength (v/v) ethanol, centrifuged for 10 minutes at 2000xg at room temperature and then dried using acetone.

10 mg of amylopectin are subsequently stirred for 10 minutes at 70°C in 250 µl of 90% strength (v/v) DMSO. 375 µl of water at a temperature of 80°C are added to the

10   solution until dissolution is complete.

200 µl of this solution are treated with 300 µl of a 16.6 mM sodium acetate solution pH 3.5 and 2 µl of isoamylase (0.24 u/µl, Megazyme, Sydney, Australia) and the mixture is incubated for 15 hours at 37°C.

A 1:4 dilution of this aqueous isoamylase reaction mixture with DMSO, comprising

15   90 mM sodium nitrate, is subsequently filtered through a 0.2 µm filter, and 24 µl of the filtrate is analysed chromatographically. Separation was carried out with two columns connected in series, first a Gram PSS3000 (Polymer Standards Service, with suitable precolumn), followed by a Gram PSS100. Detection was by means of refraction index detector (RI 71, Shodex). The column was equilibrated with DMSO

20   comprising 90 mM sodium nitrate. It was eluted with DMSO comprising 90 mM sodium nitrate at a flow rate of 0.7 ml/min over a period of 1 hour.

To correlate the elution volume with the molecular mass, the column used was calibrated with dextran standards. The dextrans used, their molecular mass and the elution volumes are shown in Table 6. Using the resulting calibration graph, the

25   elution diagram was pictured as a molecular weight distribution.

The chromatograms obtained were further evaluated using the program Wingpc Version 6 from Polymer Standards Service GmbH, Mainz, Germany.

The total area under the line of the GPC chromatogram was divided into individual segments, each of which represent groups of side chains of different lengths. The

30   chosen segments contained glucan chains with the following degree of polymerization (DP = number of glucose monomers within one side chain): DP<12, DP12-18, DP19-24, DP25-30, DP31-36, DP37-42, DP43-48, DP49-55, DP56-61 and DP62-123. To determine the molecular weight of the individual side chains, a

molecular weight of 162 was assumed for glucose. The total area under the line in the GPC chromatogram was then set as 100%, and the percentage of the areas of the individual segments was calculated based on the percentage of the total area. Results obtained from this analysis are shown in Table 7.

elution volume [ml]	molar mass [D]	sample
18,76	401300	Dextran T670
19,41	276500	Dextran T410
20,49	196300	Dextran T270
21,35	123600	Dextran T150
22,45	66700	Dextran T80
23,52	43500	Dextran T50
25,15	21400	Dextran T25
26,92	9890	Dextran T12
28,38	4440	Dextran T5
30,77	1080	Dextran T1

Table 6: Calibration table.

degree of polymerisation	% total area					
	cv.Desiree	369 SO 48	369 SO 50	369 SO 52	369 SO 106	369 SO 129
<dp12	16,49	16,57	17,07	17,73	17,59	17,64
dp12-19	13,89	14,47	14,22	14,82	14,16	14,23
dp20-25	15,74	16,54	16,15	16,74	16,32	16,51
dp26-31	9,41	9,73	9,57	9,80	9,86	9,88
dp32-37	8,53	8,53	8,45	8,56	8,59	8,45
dp38-43	6,82	6,67	6,57	6,63	6,58	6,44
dp44-49	6,05	5,91	5,81	5,83	5,79	5,72
dp50-56	4,88	4,78	4,71	4,66	4,70	4,67
dp57-62	4,26	4,15	4,10	3,98	4,09	4,10
dp63-123	13,92	12,66	13,35	11,25	12,31	12,38

Table 7: Side chain profiles DP<12, DP 12 to 18, DP 19 to 24, DP 25 to 30, DP 31 to 36, DP 37 to 42, DP 43-48, DP 49 to 55, DP 56 to 61 and DP 62 to 123 for amylopectin isolated from wild-type plants (cv. Desiree) and from plants with a reduced activity of a BEIII protein (369SO).

degree of polymerisation	% WT					
	cv.Desiree	369 SO 48	369 SO 50	369 SO 52	369 SO 106	369 SO 129
<dp12	100,00	100,47	103,53	107,53	106,69	106,96
dp12-19	100,00	104,13	102,37	106,68	101,90	102,40
dp20-25	100,00	105,11	102,62	106,32	103,69	104,87
dp26-31	100,00	103,34	101,67	104,09	104,81	104,92
dp32-37	100,00	100,01	99,05	100,38	100,73	99,04
dp38-43	100,00	97,74	96,40	97,23	96,54	94,48
dp44-49	100,00	97,68	96,00	96,27	95,76	94,48
dp50-56	100,00	97,90	96,45	95,57	96,26	95,78
dp57-62	100,00	97,36	96,07	93,44	95,91	96,13
dp63-123	100,00	90,95	95,89	80,82	88,41	88,89

Table 8: Side chain profiles DP<12, DP 12 to 18, DP 19 to 24, DP 25 to 30, DP 31 to 36, DP 37 to 42, DP 43-48, DP 49 to 55, DP 56 to 61 and DP 62 to 123 for

amylopectin isolated from wild-typ   nts (cv. Desiree) and from plants with a reduced activity of a BEIII protein (369SO). The percentages indicate the modification of the individual side chain profiles based on amylopectin isolated from wild-type plants.

5

e)   DSC-analysis („Differential Scanning Calorimetry")

Investigations with the aid of DSC-analysis („Differential Scanning Calorimetry") have been done by the method described by WO 01/19975. Results obtained from this

10   analysis are shown in Table 9.

	TO (°C)	TO (%)	T Peak (°C)	T Peak (%)	dH (J/g)	dH (J/g)
cv.Desiree	64,84	100	68,09	100	20,31	100
369SO048	64,32	99,2	67,16	98,6	20,33	100,1
369SO050	63,35	97,7	66,75	98,0	20,63	101,6
369SO052	63,27	97,6	66,46	97,6	21,23	104,5
369SO106	63,77	98,3	66,96	98,3	21,42	105,5
369SO129	63,75	98,3	67,41	99,0	20,57	101,3

Table 9: Parameters of the DSC analysis of starch isolated from wild-type plants (cv. Desiree), plants with a reduced activity of a BEIII protein (369SO) indicated in °C respectively J/g and in per cent based on data of starch of the wild type. The DSC

15   analysis was carried out as described in general methods. TO [°C] = peak onset, T Peak [°C] = Peak temperature, dH [J/g] = heat of melting.

Patent claims

1. Genetically modified plant cell, characterised in that it has a reduced activity of at least one Class 3 branching enzyme in comparison with corresponding wild type plant cells that have not been genetically modified.

2. Genetically modified plant cell according to Claim 1, wherein the genetic modification consists in the introduction of at least one foreign nucleic acid molecule into the genome of the plant cell.

3. Genetically modified plant cell according to Claim 2, wherein the foreign nucleic acid molecule codes a Class 3 branching enzyme.

4. Genetically modified plant cell according to Claim 3, wherein the said foreign nucleic acid molecule is chosen from the group consisting of

    a) Nucleic acid molecules, which code a protein with the amino acid sequence given under Seq ID No. 4;

    b) Nucleic acid molecules, which code a protein, the amino acid sequence of which has an identity of at least 50% with the amino acid sequence given under SEQ ID NO: 4;

    c) Nucleic acid molecules, which include the nucleotide sequence shown under Seq ID No. 3 or a complimentary sequence;

    d) Nucleic acid molecules, the nucleic acid sequence of which has an identity of at least 50% with the nucleic acid sequences described under a) or c);

    e) Nucleic acid molecules, which hybridise with at least one strand of the nucleic acid molecules described under a) or c) under stringent conditions;

    f) Nucleic acid molecules, the nucleotide sequence of which deviates from the sequence of the nucleic acid molecules identified under a), b), c), d), e) or f) due to the degeneration of the genetic code; and

g)   Nucleic acid molecules, which represent fragments, allelic variants and/or derivatives of the nucleic acid molecules identified under a), b), c), d), e) or f).

5.   Genetically modified plant cell according to one of Claims 2, 3 or 4, wherein the said foreign nucleic acid molecule is chosen from the group consisting of

a)   DNA molecules, which code at least one antisense RNA, which effects a reduction in the expression of at least one endogenous gene, which codes a Class 3 branching enzyme;

b)   DNA molecules, which by means of a co-suppression effect lead to the reduction in the expression of at least one endogenous gene, which codes a Class 3 branching enzyme;

c)   DNA molecules, which code at least one ribozyme, which splits specific transcripts of at least one endogenous gene, which codes a Class 3 branching enzyme;

d)   DNA molecules, which simultaneously code at least one antisense RNA and at least one sense RNA, wherein the said antisense RNA and the said sense RNA form a double-stranded RNA molecule, which effects a reduction in the expression of at least one endogenous gene, which codes a Class 3 branching enzyme (RNAi technology);

e)   Nucleic acid molecules introduced by means of in vivo mutagenesis, which lead to a mutation or an insertion of a heterologous sequence in at least one endogenous gene coding a Class 3 branching enzyme, wherein the mutation or insertion effects a reduction in the expression of a gene coding a Class 3 branching enzyme or results in the synthesis of inactive Class 3 branching enzymes;

f)   Nucleic acid molecules, which code an antibody, wherein the antibody results in a reduction in the activity of a Class 3 branching enzyme due to the bonding to a Class 3 branching enzyme.

g)   DNA molecules, which contain transposons, wherein the integration of these transposons leads to a mutation or an insertion in at least one endogenous

gene coding a Class 3 branching enzyme, which effects a reduction in the expression of at least one gene coding a Class 3 branching enzyme, or results in the synthesis of inactive Class 3 branching enzymes; and/or

h)   T-DNA molecules, which, due to insertion in at least one endogenous gene coding a Class 3 branching enzyme, effect a reduction in the expression of at least one gene coding a Class 3 branching enzyme, or result in the synthesis of inactive Class 3 branching enzyme.

6.   Plant cell according to one of Claims 1 to 5, which synthesises a modified starch in comparison with corresponding wild type plant cells that have not been genetically modified.

7.   Plant containing plant cells according to one of Claims 1 to 6.

8.   Plant according to Claim 7, which is a starch-storing plant.

9.   Plant according to Claim 8, which is a maize, rice, wheat, rye, oat, barley, cassava, potato, sago, mung bean, pea or sorghum plant.

10. Plant according to Claim 8, which is a potato plant.

11. Propagation material of plants according to one of Claims 7 to 10, containing plant cells according to one of Claims 1 to 6.

12. Harvestable plant parts of plants according to one of Claims 7 to 10, containing plant cells according to one of Claims 1 to 6.

13. Method for the manufacture of a genetically modified plant according to one of Claims 7 to 10, wherein

a)   a plant cell is genetically modified, whereby the genetic modification leads to the reduction of the activity of a Class 3 vegetable branching enzyme in comparison with corresponding wild type plant cells that have not been genetically modified;

b)   a plant is regenerated from plant cells from Step a); and

c)   if necessary, further plants are produced with the help of the plants according to Step b).

14. Method according to Claim 13, wherein the genetic modification consists in the introduction of at least one foreign nucleic acid molecule into the genome of the plant.

15. Method according to Claim 14, wherein the said foreign nucleic acid molecule is chosen from the group consisting of

a)   Nucleic acid molecules, which code a protein with the amino acid sequence given under Seq ID No. 4;

b)   Nucleic acid molecules, which code a protein, the amino acid sequence of which has an identity of at least 50% with the amino acid sequence given under SEQ ID NO: 4;

c)   Nucleic acid molecules, which include the nucleotide sequence shown under Seq ID No. 3 or a complimentary sequence;

d)   Nucleic acid molecules, the nucleic acid sequence of which has an identity of at least 50% with the nucleic acid sequences described under a) or c);

e)   Nucleic acid molecules, which hybridise with at least one strand of the nucleic acid molecules described under a) or c) under stringent conditions;

f)   Nucleic acid molecules, the nucleotide sequence of which deviates from the sequence of the nucleic acid molecules identified under a), b), c), d), e) or f) due to the degeneration of the genetic code; and

g)   Nucleic acid molecules, which represent fragments, allelic variants and/or derivatives of the nucleic acid molecules identified under a), b), c), d), e) or f).

16. Method according to Claim 14, wherein the said foreign nucleic acid molecule is chosen from the group consisting of

a)   DNA molecules, which code at least one antisense RNA, which effects a reduction in the expression of at least one endogenous gene, which codes a Class 3 branching enzyme;

b)   DNA molecules, which by means of a co-suppression effect lead to the reduction in the expression of at least one endogenous gene, which codes a Class 3 branching enzyme;

c)   DNA molecules, which code at least one ribozyme, which splits specific transcripts of at least one endogenous gene, which codes a Class 3 branching enzyme;

d)   DNA molecules, which simultaneously code at least one antisense RNA and at least one sense RNA, wherein the said antisense RNA and the said sense RNA form a double-stranded RNA molecule, which effects a reduction in the expression of at least one endogenous gene, which codes a Class 3 branching enzyme (RNAi technology);

e)   Nucleic acid molecules introduced by means of in vivo mutagenesis, which lead to a mutation or an insertion of a heterologous sequence in at least one endogenous gene coding a Class 3 branching enzyme, wherein the mutation or insertion effects a reduction in the expression of a gene coding a Class 3 branching enzyme or results in the synthesis of inactive Class 3 branching enzymes;

f)   Nucleic acid molecules, which code an antibody, wherein the antibody results in a reduction in the activity of a Class 3 branching enzyme due to the bonding to a Class 3 branching enzyme.

g)   DNA molecules, which contain transposons, wherein the integration of these transposons leads to a mutation or an insertion in at least one endogenous gene coding a Class 3 branching enzyme, which effects a reduction in the expression of at least one gene coding a Class 3 branching enzyme, or results in the synthesis of inactive Class 3 branching enzymes; and/or

76

h) T-DNA molecules, which, due to insertion in at least one endogenous gene coding a Class 3 branching enzyme, effect a reduction in the expression of at least one gene coding a Class 3 branching enzyme, or result in the synthesis of inactive Class 3 branching enzyme.

17. Method according to one of Claims 13 to 17 [sic], wherein the genetically modified plant synthesises a modified starch in comparison with corresponding wild type plants that have not been genetically modified.

18. Nucleic acid molecule, coding for a protein with the enzymatic activity of a Class 3 branching enzyme, chosen from the group consisting of

a) Nucleic acid molecules, which code a protein with the amino acid sequence given under Seq ID No. 4;

b) Nucleic acid molecules, which code a protein, the amino acid sequence of which has an identity of at least 70% with the amino acid sequence given under SEQ ID NO: 4;

c) Nucleic acid molecules, which include the nucleotide sequence shown under Seq ID No. 3 or a complimentary sequence;

d) Nucleic acid molecules, which have an identity of at least 70% with the nucleic acid sequences described under a) or c);

e) Nucleic acid molecules, which hybridise with at least one strand of the nucleic acid molecules described under a) or c) under stringent conditions;

f) Nucleic acid molecules, the nucleotide sequence of which deviates from the sequence of the nucleic acid molecules identified under a), b), c), d), e) or f) due to the degeneration of the genetic code; and

g) Nucleic acid molecules, which represent fragments, allelic variants and/or derivatives of the nucleic acid molecules identified under a), b), c), d), e) or f).

19. Nucleic acid molecule according to Claim 18, characterised in that it codes a Class 3 branching enzyme of potato.

20. Vector containing a nucleic acid molecule according to one of Claims 18 or 19.

21. Vector according to Claim 20, wherein the nucleic acid molecule is linked with regulatory sequences, which guarantee transcription into prokaryontic or eukaryontic cells.

22. Vector containing a foreign nucleic acid molecule defined as in Claim 5 under a), b), c) or d).

23. Host cell, which is genetically modified with a nucleic acid molecule according to one of Claims 18 or 19 or with a vector according to one of Claims 20, 21 or 22.

24. Protein with the enzymatic activity of a Class 3 branching enzyme, chosen from the group consisting of

   a)   Proteins, which include the amino acid sequence specified under SEQ ID No. 4, or

   b)   Proteins, which have an identity of at least 70% with the amino acid sequence of the proteins identified under a).

25. Protein according to Claim 24, wherein the Class 3 branching enzyme comes from a potato plant.

26. Modified starch obtainable from a genetically modified plant according to one of Claims 7 to 10, from propagation material according to Claim 11, or from harvestable plant parts according to Claim 12.

27. Method for the manufacture of a modified starch including the step of extracting the starch from a plant cell according to one of Claims 1 to 6.

28. Method for the manufacture of a modified starch including the step of extracting the starch from a plant according to one of Claims 7 to 10, and/or from starch-storing parts of such a plant.

29. Method for the manufacture of a modified starch including the step of extracting the starch from harvestable plant parts according to Claim 12.

30. Method for the manufacture of a derived starch, wherein modified starch according to Claim 26 or obtainable by means of a method according to one of Claims 27, 28 or 29 is derived.

31. Use of genetically modified plants according to one of Claims 7 to 10 for the manufacture of a modified starch.

32. Modified starch obtainable by means of a method according to one of Claims 27, 28 or 29.

33. Derived starch obtainable by means of a method according to Claim 30.

34. Use of modified starch according to one of Claims 26 or 32 for the manufacture of derived starch.

BCS 03-5004_SEQUENZPROTOKOLL.ST25
SEQUENCE LISTING

<110> Bayer CropScience GmbH

<120> Plants with reduced activity of a Class 3 branching enzyme

<130> BCS 03-5004-PCT

<150> EP 03090325.62003

<151> 2003-09-30

<160> 6

<170> PatentIn version 3.1

<210> 1

<211> 1004

<212> DNA

<213> Solanum tuberosum

<400> 1
tcaaactagt cacaaccagt ccatttctgg aggtcgttcc ttcgcagaaa tactgattgg   60

taactccttg gggaaatcct ccatatcaca agagtcatta cttagaggct gctcgttaca   120

caagatgatc agattaatta catctacaat tggtggtcat gcatacctca acttcatggg   180

caatgaattt ggtcacccaa agagagtaga gtttccaatg tcaagcaaca atttctcctt   240

ttcactggct aaccgtcgct gggatctatt ggaagatgtt gtacattatc aattgttctc   300

atttgataag ggtatgatgg acttggataa aaatgggaga attttgtcca gaggtcttgc   360

caacattcac catgtcaatg atactaccat ggtgatttct tacttgagag gtcccaatct   420

ctttgtgttc aactttcatc ctgtcaattc atatgaaaga tacattatag gtgtggaaga   480

agctggagag tatcaagtca cattaaatac agatgaaaac aagtatggtg gtagaggact   540

acttggccat gatcagaata ttcaaagaac cattagtaga agagctgatg gaatgagatt   600

ttgcttggaa gtgcctctgc caagtagaag tgctcaggtc tacaagttga cccgaattct   660

aagagcatga tcactctagt aatcaaagtg cctcatatga tgacacaaaa ggaaaggttc   720

tacattgccc ttacactgat caatattgac acctttccga ggtgagtttc tgtgattctt   780

gagcagactg ttggctagtc aattatcatg aacttttgcc ttcagcatcc ggatagtcgc   840

BCS 03-5004_SEQUENZPROTOKOLL.ST25

```
ttctcctgtg caatgagggc atggacgaat ttttttttgg cttgtcatgg gggtcataag    900

catccgccag attaagattt cacaggcctc gagtaaaacc atcacttact ttaaggatac    960

acaaacacac caacggggtg caggctctga taccttctaa agtg                     1004
```

<210> 2

<211> 2096

<212> DNA

<213> Solanum tuberosum

<400> 2
```
aacaatgctc tctctgtcgg attcaattcg aatttcttca ccattgagcg attctcgtct     60

tagtttttcta tctcaaaccg gaagcagaac cagtcgccag cttaaatttg ttcgcagccg    120

ccgggctcga gtttcgaggt gtagatgctc agcaacggag caaccgccac cgcaacgacg    180

gaagcaacga ccggagaagt acaaacagtc ggaggaaggg aaaggaatcg atcctgttgg    240

atttctcagc aaatacggca ttactcataa agcgtttgct caatttcttc gtgaaagata    300

taaatcattg aaggacttga aggatgaaat attgactcgt catttcagtc tcaaggagat    360

gtctactggg tatgaattaa tgggtatgca tcgcaacata caacatcgag tggatttctt    420

ggaatgggct ccaggtgctc gctactgtgc tctgattggt gacttcaatg ggtggtcaac    480

aactggtaac tgtgccagag agggtcattt tggtcatgac gattatgggt attggtttat    540

tattcttgaa gataaattac gtgaaggaga agaacctgat aaattgtatt ttcaacagta    600

caattatgcg gaggactatg gtaaaggtga cacgggtatt accgtcgagg aaatctttaa    660

aaaagcaaat gatgagtatt gggaacctgg agaagatcgc ttcattaaat cacgttatga    720

ggtggcagca aagttatatg aggaaatgtt cggaccaaat ggacctcaaa cagaagagga    780

actagaagca atgcctgatg cagctacacg atacaaaact tggaaagagc aacaaaaaga    840

ggatccggca agcaatttgc catcgtatga tgtggtagat agtggaaaag aatatgatat    900

ttacaatatt ataggtgatc ctgaatcgtt taagaaattt cgtatgaaac agcctcctat    960

tgcttactgg ttagaaacta aaaagggaag gaaaggctgg ttacagaaat atatgcctgc   1020

tttacctcat ggaagcaaat acagggtgta ttttaacaca ccaaatgggc ctccttgaacg  1080

agttcctgcg tgggccaatt ttgtcattcc agatgcaggc gggatggcat tagcagtcca   1140

ttgggaacca cctcctgaat atgcttataa atggaaacac aagctaccag tcaagcctaa   1200

gtccttgcgc atatatgaat gtcatgttgg catctctggc caggaaccaa aagtttcatc   1260

tttcaatgat tttattagca aggtccttcc gcatgtaaaa gaagctggat acaatgcaat   1320

acaaattatt ggagttgttg agcacaagga ttatttcact gttggatata gagtgaccaa   1380

ttttttatgct gttagtagcc gttatggcac accggatgac ttcaagcgct tggttgatga   1440

agcacatggg cttggactgc ttgtcttttt ggagattgtg cactcttatg cagcagcaga   1500
```

BCS 03-5004_SEQUENZPROTOKOLL.ST25

```
tgaaatggtt gggttatctc tttttgatgg agcaaatgat tgctatttcc acactggtaa   1560

acgtggacac cacaaattct ggggcacacg gatgttcaaa tatggagatc ttgatgttct   1620

gcactttctt ctttcaaatc tgaactggtg ggtggaggag tatcatgtcg atggcttcca   1680

ttttcattcg ctctcgtcca tgttgtatac gcataatgga tttgcttcat ttactggtga   1740

catggatgaa tactgtaacc aatatgttga caaggaggcc ttattgtacc tcatattagc   1800

aaatgaagta ttacatgctc ttcatcctaa tgtgatcacg attgctgagg atgcaactct   1860

gtatcctgga ctctgcgatc caacatctca aggtggactg ggctttgatt attttgccaa   1920

tctttctgcc tcagagatgt ggcttgcatt acttgaaaat actcctgatc atgaatggtg   1980

catgagtaag attgttagca cattagtggg cgatagacaa aatactgata aaatgctttt   2040

gtatgcagaa aatcacaacc agtccatttc tggaggtcgt tccttcgcag aaatac       2096
```

<210>　3

<211>　3204

<212>　DNA

<213>　Solanum tuberosum

<220>

<221>　CDS

<222>　(99)..(2804)

<223>


```
<400>  3
gaattgtaat acgactcact atagggcgaa ttgggccctc tagatgcatg ctcgagcggc     60

cgccagtgtg atggatatct gcagaattcg cttaaca atg ctc tct ctg tcg gat    116
                                           Met Leu Ser Leu Ser Asp
                                           1               5

tca att cga att tct tca cca ttg agc gat tct cgt ctt agt ttt cta    164
Ser Ile Arg Ile Ser Ser Pro Leu Ser Asp Ser Arg Leu Ser Phe Leu
         10                  15                  20

tct caa acc gga agc aga acc agt cgc cag ctt aaa ttt gtt cgc agc    212
Ser Gln Thr Gly Ser Arg Thr Ser Arg Gln Leu Lys Phe Val Arg Ser
         25                  30                  35

cgc cgg gct cga gtt tcg agg tgt aga tgc tca gca acg gag caa ccg    260
Arg Arg Ala Arg Val Ser Arg Cys Arg Cys Ser Ala Thr Glu Gln Pro
         40                  45                  50

cca ccg caa cga cgg aag caa cga ccg gag aag tac aaa cag tcg gag    308
Pro Pro Gln Arg Arg Lys Gln Arg Pro Glu Lys Tyr Lys Gln Ser Glu
55                  60                  65                  70

gaa gag aaa gga atc gat cct gtt gga ttt ctc agc aaa tac ggc att    356
Glu Glu Lys Gly Ile Asp Pro Val Gly Phe Leu Ser Lys Tyr Gly Ile
                 75                  80                  85
```

BCS 03-5004_SEQUENZPROTOKOLL.ST25

```
act cat aaa gcg ttt gct caa ttt ctt cgt gaa aga tat aaa tca ttg    404
Thr His Lys Ala Phe Ala Gln Phe Leu Arg Glu Arg Tyr Lys Ser Leu
        90              95                  100

aag gac ttg aag gat gaa ata ttg act cgt cat ttc agt ctc aag gag    452
Lys Asp Leu Lys Asp Glu Ile Leu Thr Arg His Phe Ser Leu Lys Glu
        105             110                 115

atg tct act ggg tat gaa tta atg ggt atg cat cgc aac ata caa cat    500
Met Ser Thr Gly Tyr Glu Leu Met Gly Met His Arg Asn Ile Gln His
        120             125                 130

cga gtg gat ttc ttg gaa tgg gct cca ggt gct cgc tac tgt gct ctg    548
Arg Val Asp Phe Leu Glu Trp Ala Pro Gly Ala Arg Tyr Cys Ala Leu
135             140                 145                 150

att ggt gac ttc aat ggg tgg tca aca act ggt aac tgt gcc aga gag    596
Ile Gly Asp Phe Asn Gly Trp Ser Thr Thr Gly Asn Cys Ala Arg Glu
                155                 160                 165

ggt cat ttt ggt cat gac gat tat ggg tat tgg ttt att att ctt gaa    644
Gly His Phe Gly His Asp Asp Tyr Gly Tyr Trp Phe Ile Ile Leu Glu
        170                 175                 180

gat aaa tta cgt gaa gga gaa gaa cct gat aaa ttg tat ttt caa cag    692
Asp Lys Leu Arg Glu Gly Glu Glu Pro Asp Lys Leu Tyr Phe Gln Gln
        185                 190                 195

tac aat tat gcg gag gac tat gat aaa ggt gac acg ggt att acc gtc    740
Tyr Asn Tyr Ala Glu Asp Tyr Asp Lys Gly Asp Thr Gly Ile Thr Val
        200                 205                 210

gag gaa atc ttt aaa aaa gca aat gat gag tat tgg gaa cct gga gaa    788
Glu Glu Ile Phe Lys Lys Ala Asn Asp Glu Tyr Trp Glu Pro Gly Glu
215                 220                 225                 230

gat cgc ttc att aaa tca cgt tat gag gtg gca gca aag tta tat gag    836
Asp Arg Phe Ile Lys Ser Arg Tyr Glu Val Ala Ala Lys Leu Tyr Glu
                235                 240                 245

gaa atg ttc gga cca aat gga cct caa aca gaa gag gaa cta gaa gca    884
Glu Met Phe Gly Pro Asn Gly Pro Gln Thr Glu Glu Glu Leu Glu Ala
        250                 255                 260

atg cct gat gca gct aca cga tac aaa act tgg aaa gag caa caa aaa    932
Met Pro Asp Ala Ala Thr Arg Tyr Lys Thr Trp Lys Glu Gln Gln Lys
        265                 270                 275

aag gat ccg gca agc aat ttg cca tcg tat gat gtg gta gat agt gga    980
Lys Asp Pro Ala Ser Asn Leu Pro Ser Tyr Asp Val Val Asp Ser Gly
        280                 285                 290

aaa gaa tat gat att tac aat att ata ggt gat cct gaa tcg ttt aag    1028
Lys Glu Tyr Asp Ile Tyr Asn Ile Ile Gly Asp Pro Glu Ser Phe Lys
295                 300                 305                 310

aaa ttt cgt atg aaa cag cct cct att gct tac tgg tta gaa act aaa    1076
Lys Phe Arg Met Lys Gln Pro Pro Ile Ala Tyr Trp Leu Glu Thr Lys
                315                 320                 325

aag gga agg aaa ggc tgg tta cag aaa tat atg cct gct tta cct cat    1124
Lys Gly Arg Lys Gly Trp Leu Gln Lys Tyr Met Pro Ala Leu Pro His
        330                 335                 340

gga agc aaa cac agg gtg tat ttt aac aca cca aat ggg cct ctt gaa    1172
Gly Ser Lys His Arg Val Tyr Phe Asn Thr Pro Asn Gly Pro Leu Glu
        345                 350                 355
```

BCS 03-5004_SEQUENZPROTOKOLL.ST25

```
cga gtt cct gcg tgg gcc aat ttt gtc att cca gat gca gac ggg atg    1220
Arg Val Pro Ala Trp Ala Asn Phe Val Ile Pro Asp Ala Asp Gly Met
    360             365             370

gca tta gca gtc cat tgg gaa cca cct cct gaa tat gct tat aaa tgg    1268
Ala Leu Ala Val His Trp Glu Pro Pro Pro Glu Tyr Ala Tyr Lys Trp
375             380             385             390

aaa cac aag cta cca gtc aag cct aag tcc ttg cgc ata tat gaa tgt    1316
Lys His Lys Leu Pro Val Lys Pro Lys Ser Leu Arg Ile Tyr Glu Cys
                395             400             405

cat gtt ggc atc tct ggc cag gaa cca aaa gtt tca tct ttc aat gat    1364
His Val Gly Ile Ser Gly Gln Glu Pro Lys Val Ser Ser Phe Asn Asp
            410             415             420

ttt att agc aag gtc ctt ccg cat gta aaa gaa gct gga tac aat gca    1412
Phe Ile Ser Lys Val Leu Pro His Val Lys Glu Ala Gly Tyr Asn Ala
            425             430             435

acg caa att att gga gtt gtt gag cac aag gat tat ttc act gtt gga    1460
Thr Gln Ile Ile Gly Val Val Glu His Lys Asp Tyr Phe Thr Val Gly
            440             445             450

tat aga gtg acc aat ttt tat gct gtt agt agc cgt tat ggc aca ccg    1508
Tyr Arg Val Thr Asn Phe Tyr Ala Val Ser Ser Arg Tyr Gly Thr Pro
455             460             465             470

gat gac ttc aag cgc ttg gtt gat gaa gca cat ggg ctt gga ctg ctt    1556
Asp Asp Phe Lys Arg Leu Val Asp Glu Ala His Gly Leu Gly Leu Leu
                475             480             485

gtc ttt ttg gag att gtg cac tcc tat gca gca gca gat gaa atg gtt    1604
Val Phe Leu Glu Ile Val His Ser Tyr Ala Ala Ala Asp Glu Met Val
            490             495             500

ggg tta tct ctt ttt gat gga gca aat gat tgc tat ttc cac act ggt    1652
Gly Leu Ser Leu Phe Asp Gly Ala Asn Asp Cys Tyr Phe His Thr Gly
        505             510             515

aaa cgt gga cac cac aaa ttc tgg ggc aca cgg atg ttc aaa tat gga    1700
Lys Arg Gly His His Lys Phe Trp Gly Thr Arg Met Phe Lys Tyr Gly
        520             525             530

gat cct gat gtt ctg cac ttt ctt ctt tca aat ctg aac tgg tgg gtg    1748
Asp Pro Asp Val Leu His Phe Leu Leu Ser Asn Leu Asn Trp Trp Val
535             540             545             550

gag gag tat cat gtc gat ggc ttc cat ttt cat tcg ctc tcg tcc atg    1796
Glu Glu Tyr His Val Asp Gly Phe His Phe His Ser Leu Ser Ser Met
                555             560             565

ttg tat acg cat aat gga ttt gct tca ttt act ggt gac atg gat gaa    1844
Leu Tyr Thr His Asn Gly Phe Ala Ser Phe Thr Gly Asp Met Asp Glu
            570             575             580

tac tgt aac caa tat gtt gac aag gag gcc tta ttg tac ctc ata tta    1892
Tyr Cys Asn Gln Tyr Val Asp Lys Glu Ala Leu Leu Tyr Leu Ile Leu
            585             590             595

gca aat gaa gta tta cat gct ctt cat cct aat gtg atc acg att gct    1940
Ala Asn Glu Val Leu His Ala Leu His Pro Asn Val Ile Thr Ile Ala
    600             605             610

gtg gat gca act ctg tat cct gga ctc tgc gat cca aca tct caa ggt    1988
Val Asp Ala Thr Leu Tyr Pro Gly Leu Cys Asp Pro Thr Ser Gln Gly
615             620             625             630
```

BCS 03-5004_SEQUENZPROTOKOLL.ST25

```
gga ctg ggc ttt gat tat ttt gcc aat ctt tct gcc tca gag atg tgg    2036
Gly Leu Gly Phe Asp Tyr Phe Ala Asn Leu Ser Ala Ser Glu Met Trp
            635             640             645

ctt gca tta ctt gaa aat act cct gat cat gaa tgg tgc atg agt aag    2084
Leu Ala Leu Leu Glu Asn Thr Pro Asp His Glu Trp Cys Met Ser Lys
            650             655             660

att gtt agc aca tta gtg ggc gat aga caa aat act gat aaa atg ctt    2132
Ile Val Ser Thr Leu Val Gly Asp Arg Gln Asn Thr Asp Lys Met Leu
            665             670             675

ttg tat gca gaa aat cac aac cag tcc att tct gga ggt cgt tcc ttc    2180
Leu Tyr Ala Glu Asn His Asn Gln Ser Ile Ser Gly Gly Arg Ser Phe
            680             685             690

gca gaa ata ctg att ggt aac tcc ttg ggg aaa tct tcc ata tca caa    2228
Ala Glu Ile Leu Ile Gly Asn Ser Leu Gly Lys Ser Ser Ile Ser Gln
695             700             705             710

gag tca tta ctt aga ggc tgc tcg tta cac aag atg atc aga tta att    2276
Glu Ser Leu Leu Arg Gly Cys Ser Leu His Lys Met Ile Arg Leu Ile
            715             720             725

aca tct aca att ggt ggt cat gca tac ctc aac ttc atg ggc aat gaa    2324
Thr Ser Thr Ile Gly Gly His Ala Tyr Leu Asn Phe Met Gly Asn Glu
            730             735             740

ttt ggt cac cca aag aga gta gag ttt cca atg tca agc aac aat ttc    2372
Phe Gly His Pro Lys Arg Val Glu Phe Pro Met Ser Ser Asn Asn Phe
            745             750             755

tcc ttt tca ctg gct aac cgt cgc tgg gat cta ttg gaa gat gtt gta    2420
Ser Phe Ser Leu Ala Asn Arg Arg Trp Asp Leu Leu Glu Asp Val Val
            760             765             770

cat tat caa tta ttc tca ttt gat aag gat atg atg gac ttg gat aaa    2468
His Tyr Gln Leu Phe Ser Phe Asp Lys Asp Met Met Asp Leu Asp Lys
775             780             785             790

aat ggg aga att ttg tcc aga ggt ctt gcc aac att cac cat gtc aat    2516
Asn Gly Arg Ile Leu Ser Arg Gly Leu Ala Asn Ile His His Val Asn
            795             800             805

gat act acc atg gtg att tct tac ttg aga ggt ccc aat ctc ttt gtg    2564
Asp Thr Thr Met Val Ile Ser Tyr Leu Arg Gly Pro Asn Leu Phe Val
            810             815             820

ttc aac ttt cat cct gtc aat tca tat gaa aga tac att ata ggt gtg    2612
Phe Asn Phe His Pro Val Asn Ser Tyr Glu Arg Tyr Ile Ile Gly Val
            825             830             835

gaa gaa gct gga gag tat caa gtc aca tta aat aca gat gaa aac aag    2660
Glu Glu Ala Gly Glu Tyr Gln Val Thr Leu Asn Thr Asp Glu Asn Lys
            840             845             850

tat ggt ggt aga gga cta ctt ggc cat gat cag aat act caa aga acc    2708
Tyr Gly Gly Arg Gly Leu Leu Gly His Asp Gln Asn Thr Gln Arg Thr
855             860             865             870

att agt aga aga gct gat gga atg aga ttt tgc ttg gaa gta cct ctg    2756
Ile Ser Arg Arg Ala Asp Gly Met Arg Phe Cys Leu Glu Val Pro Leu
            875             880             885

cca agt aga agt gct cag gtc tac aag ttg acc cga att cta aga gca    2804
Pro Ser Arg Ser Ala Gln Val Tyr Lys Leu Thr Arg Ile Leu Arg Ala
            890             895             900
```

BCS 03-5004_SEQUENZPROTOKOLL.ST25

```
tgatcactct agcaatcaaa gtgcctcata tgatcacaca aaagggaagg ttctacattg    2864

cccttatact gaccaatatt gtggcctttc cgaggtgagt ttctgtgatt cttgagcaca    2924

ggctgttggc tagtcagtta tcatgaactt ttgccttcag catctggata agcgcttctc    2984

ctgtgcaatg agggcatgga cgaaattttt ttggttcgtc atgggagtca aaagcatctg    3044

ccagattaag atttcacagg cctcgagtaa aaccatcact tacttaggat acacaaacac    3104

atcaacgggg tgcaggctct gataccttct aaagtgaagc cgaattccag cacactggcg    3164

gccgttacta gtggatccga gctcggtacc aagcttggcg                          3204
```

<210>   4

<211>   902

<212>   PRT

<213>   Solanum tuberosum

<400>   4

```
Met Leu Ser Leu Ser Asp Ser Ile Arg Ile Ser Ser Pro Leu Ser Asp
1               5                   10                  15


Ser Arg Leu Ser Phe Leu Ser Gln Thr Gly Ser Arg Thr Ser Arg Gln
            20                  25                  30


Leu Lys Phe Val Arg Ser Arg Arg Ala Arg Val Ser Arg Cys Arg Cys
        35                  40                  45


Ser Ala Thr Glu Gln Pro Pro Pro Gln Arg Arg Lys Gln Arg Pro Glu
    50                  55                  60


Lys Tyr Lys Gln Ser Glu Glu Glu Lys Gly Ile Asp Pro Val Gly Phe
65                  70                  75                  80


Leu Ser Lys Tyr Gly Ile Thr His Lys Ala Phe Ala Gln Phe Leu Arg
                85                  90                  95


Glu Arg Tyr Lys Ser Leu Lys Asp Leu Lys Asp Glu Ile Leu Thr Arg
            100                 105                 110


His Phe Ser Leu Lys Glu Met Ser Thr Gly Tyr Glu Leu Met Gly Met
        115                 120                 125


His Arg Asn Ile Gln His Arg Val Asp Phe Leu Glu Trp Ala Pro Gly
    130                 135                 140


Ala Arg Tyr Cys Ala Leu Ile Gly Asp Phe Asn Gly Trp Ser Thr Thr
145                 150                 155                 160
```

BCS_03-5004_SEQUENZPROTOKOLL.ST25

```
Gly Asn Cys Ala Arg Glu Gly His Phe Gly His Asp Asp Tyr Gly Tyr
            165             170             175


Trp Phe Ile Ile Leu Glu Asp Lys Leu Arg Glu Gly Glu Glu Pro Asp
            180             185             190


Lys Leu Tyr Phe Gln Gln Tyr Asn Tyr Ala Glu Asp Tyr Asp Lys Gly
            195             200             205


Asp Thr Gly Ile Thr Val Glu Glu Ile Phe Lys Lys Ala Asn Asp Glu
    210             215             220


Tyr Trp Glu Pro Gly Glu Asp Arg Phe Ile Lys Ser Arg Tyr Glu Val
225             230             235             240


Ala Ala Lys Leu Tyr Glu Glu Met Phe Gly Pro Asn Gly Pro Gln Thr
            245             250             255


Glu Glu Glu Leu Glu Ala Met Pro Asp Ala Ala Thr Arg Tyr Lys Thr
            260             265             270


Trp Lys Glu Gln Gln Lys Lys Asp Pro Ala Ser Asn Leu Pro Ser Tyr
            275             280             285


Asp Val Val Asp Ser Gly Lys Glu Tyr Asp Ile Tyr Asn Ile Ile Gly
    290             295             300


Asp Pro Glu Ser Phe Lys Lys Phe Arg Met Lys Gln Pro Pro Ile Ala
305             310             315             320


Tyr Trp Leu Glu Thr Lys Lys Gly Arg Lys Gly Trp Leu Gln Lys Tyr
            325             330             335


Met Pro Ala Leu Pro His Gly Ser Lys His Arg Val Tyr Phe Asn Thr
            340             345             350


Pro Asn Gly Pro Leu Glu Arg Val Pro Ala Trp Ala Asn Phe Val Ile
            355             360             365


Pro Asp Ala Asp Gly Met Ala Leu Ala Val His Trp Glu Pro Pro Pro
    370             375             380


Glu Tyr Ala Tyr Lys Trp Lys His Lys Leu Pro Val Lys Pro Lys Ser
385             390             395             400


Leu Arg Ile Tyr Glu Cys His Val Gly Ile Ser Gly Gln Glu Pro Lys
            405             410             415


Val Ser Ser Phe Asn Asp Phe Ile Ser Lys Val Leu Pro His Val Lys
            420             425             430
```

BCS_03-5004_SEQUENZPROTOKOLL.ST25

```
Glu Ala Gly Tyr Asn Ala Thr Gln Ile Ile Gly Val Val Glu His Lys
        435              440              445

Asp Tyr Phe Thr Val Gly Tyr Arg Val Thr Asn Phe Tyr Ala Val Ser
    450              455              460

Ser Arg Tyr Gly Thr Pro Asp Asp Phe Lys Arg Leu Val Asp Glu Ala
465              470              475              480

His Gly Leu Gly Leu Leu Val Phe Leu Glu Ile Val His Ser Tyr Ala
            485              490              495

Ala Ala Asp Glu Met Val Gly Leu Ser Leu Phe Asp Gly Ala Asn Asp
            500              505              510

Cys Tyr Phe His Thr Gly Lys Arg Gly His His Lys Phe Trp Gly Thr
        515              520              525

Arg Met Phe Lys Tyr Gly Asp Pro Asp Val Leu His Phe Leu Leu Ser
    530              535              540

Asn Leu Asn Trp Trp Val Glu Glu Tyr His Val Asp Gly Phe His Phe
545              550              555              560

His Ser Leu Ser Ser Met Leu Tyr Thr His Asn Gly Phe Ala Ser Phe
            565              570              575

Thr Gly Asp Met Asp Glu Tyr Cys Asn Gln Tyr Val Asp Lys Glu Ala
            580              585              590

Leu Leu Tyr Leu Ile Leu Ala Asn Glu Val Leu His Ala Leu His Pro
        595              600              605

Asn Val Ile Thr Ile Ala Val Asp Ala Thr Leu Tyr Pro Gly Leu Cys
    610              615              620

Asp Pro Thr Ser Gln Gly Gly Leu Gly Phe Asp Tyr Phe Ala Asn Leu
625              630              635              640

Ser Ala Ser Glu Met Trp Leu Ala Leu Leu Glu Asn Thr Pro Asp His
            645              650              655

Glu Trp Cys Met Ser Lys Ile Val Ser Thr Leu Val Gly Asp Arg Gln
            660              665              670

Asn Thr Asp Lys Met Leu Leu Tyr Ala Glu Asn His Asn Gln Ser Ile
        675              680              685

Ser Gly Gly Arg Ser Phe Ala Glu Ile Leu Ile Gly Asn Ser Leu Gly
    690              695              700
```

BCS 03-5004_SEQUENZPROTOKOLL.ST25
```
Lys Ser Ser Ile Ser Gln Glu Ser Leu Leu Arg Gly Cys Ser Leu His
705             710             715             720


Lys Met Ile Arg Leu Ile Thr Ser Thr Ile Gly Gly His Ala Tyr Leu
                725             730             735


Asn Phe Met Gly Asn Glu Phe Gly His Pro Lys Arg Val Glu Phe Pro
            740             745             750


Met Ser Ser Asn Asn Phe Ser Phe Ser Leu Ala Asn Arg Arg Trp Asp
        755             760             765


Leu Leu Glu Asp Val Val His Tyr Gln Leu Phe Ser Phe Asp Lys Asp
    770             775             780


Met Met Asp Leu Asp Lys Asn Gly Arg Ile Leu Ser Arg Gly Leu Ala
785             790             795             800


Asn Ile His His Val Asn Asp Thr Thr Met Val Ile Ser Tyr Leu Arg
            805             810             815


Gly Pro Asn Leu Phe Val Phe Asn Phe His Pro Val Asn Ser Tyr Glu
            820             825             830


Arg Tyr Ile Ile Gly Val Glu Glu Ala Gly Glu Tyr Gln Val Thr Leu
        835             840             845


Asn Thr Asp Glu Asn Lys Tyr Gly Gly Arg Gly Leu Leu Gly His Asp
    850             855             860


Gln Asn Thr Gln Arg Thr Ile Ser Arg Arg Ala Asp Gly Met Arg Phe
865             870             875             880


Cys Leu Glu Val Pro Leu Pro Ser Arg Ser Ala Gln Val Tyr Lys Leu
            885             890             895


Thr Arg Ile Leu Arg Ala
            900
```

<210> 5

<211> 3047

<212> DNA

<213> Solanum tuberosum

<220>

<221> CDS

<222> (5)..(2710)

BCS 03-5004_SEQUENZPROTOKOLL.ST25
<223>

<400> 5
```
aaca atg ctc tct ctg tcg gat tca att cga att tct tca cca ttg agc     49
     Met Leu Ser Leu Ser Asp Ser Ile Arg Ile Ser Ser Pro Leu Ser
     1               5                   10                  15

gat tct cgt ctt agt ttt cta tct caa acc gga agc aga acc agt cgc     97
Asp Ser Arg Leu Ser Phe Leu Ser Gln Thr Gly Ser Arg Thr Ser Arg
             20              25                  30

cag ctt aaa ttt gtt cgc agc cgc cgg gct cga gtt tcg agg tgt aga    145
Gln Leu Lys Phe Val Arg Ser Arg Arg Ala Arg Val Ser Arg Cys Arg
         35              40                  45

tgc tca gca acg gag caa ccg cca ccg caa cga cgg aag caa cga ccg    193
Cys Ser Ala Thr Glu Gln Pro Pro Pro Gln Arg Arg Lys Gln Arg Pro
         50              55                  60

gag aag tac aaa cag tcg gag gaa ggg aaa gga atc gat cct gtt gga    241
Glu Lys Tyr Lys Gln Ser Glu Glu Gly Lys Gly Ile Asp Pro Val Gly
     65                  70                  75

ttt ctc agc aaa tac ggc att act cat aaa gcg ttt gct caa ttt ctt    289
Phe Leu Ser Lys Tyr Gly Ile Thr His Lys Ala Phe Ala Gln Phe Leu
80                  85                  90                  95

cgt gaa aga tat aaa tca ttg aag gac ttg aag gat gaa ata ttg act    337
Arg Glu Arg Tyr Lys Ser Leu Lys Asp Leu Lys Asp Glu Ile Leu Thr
             100                 105                 110

cgt cat ttc agt ctc aag gag atg tct act ggg tat gaa tta atg ggt    385
Arg His Phe Ser Leu Lys Glu Met Ser Thr Gly Tyr Glu Leu Met Gly
             115                 120                 125

atg cat cgc aac ata caa cat cga gtg gat ttc ttg gaa tgg gct cca    433
Met His Arg Asn Ile Gln His Arg Val Asp Phe Leu Glu Trp Ala Pro
         130                 135                 140

ggt gct cgc tac tgt gct ctg att ggt gac ttc aat ggg tgg tca aca    481
Gly Ala Arg Tyr Cys Ala Leu Ile Gly Asp Phe Asn Gly Trp Ser Thr
         145                 150                 155

act ggt aac tgt gcc aga gag ggt cat ttt ggt cat gac gat tat ggg    529
Thr Gly Asn Cys Ala Arg Glu Gly His Phe Gly His Asp Asp Tyr Gly
160                 165                 170                 175

tat tgg ttt att att ctt gaa gat aaa tta cgt gaa gga gaa gaa cct    577
Tyr Trp Phe Ile Ile Leu Glu Asp Lys Leu Arg Glu Gly Glu Glu Pro
             180                 185                 190

gat aaa ttg tat ttt caa cag tac aat tat gcg gag gac tat ggt aaa    625
Asp Lys Leu Tyr Phe Gln Gln Tyr Asn Tyr Ala Glu Asp Tyr Gly Lys
             195                 200                 205

ggt gac acg ggt att acc gtc gag gaa atc ttt aaa aaa gca aat gat    673
Gly Asp Thr Gly Ile Thr Val Glu Glu Ile Phe Lys Lys Ala Asn Asp
         210                 215                 220

gag tat tgg gaa cct gga gaa gat cgc ttc att aaa tca cgt tat gag    721
Glu Tyr Trp Glu Pro Gly Glu Asp Arg Phe Ile Lys Ser Arg Tyr Glu
225                 230                 235

gtg gca gca aag tta tat gag gaa atg ttc gga cca aat gga cct caa    769
Val Ala Ala Lys Leu Tyr Glu Glu Met Phe Gly Pro Asn Gly Pro Gln
240                 245                 250                 255
```

BCS 03-5004_SEQUENZPROTOKOLL.ST25

```
aca gaa gag gaa cta gaa gca atg cct gat gca gct aca cga tac aaa    817
Thr Glu Glu Glu Leu Glu Ala Met Pro Asp Ala Ala Thr Arg Tyr Lys
            260                 265                 270

act tgg aaa gag caa caa aaa gag gat ccg gca agc aat ttg cca tcg    865
Thr Trp Lys Glu Gln Gln Lys Glu Asp Pro Ala Ser Asn Leu Pro Ser
            275                 280                 285

tat gat gtg gta gat agt gga aaa gaa tat gat att tac aat att ata    913
Tyr Asp Val Val Asp Ser Gly Lys Glu Tyr Asp Ile Tyr Asn Ile Ile
            290                 295                 300

ggt gat cct gaa tcg ttt aag aaa ttt cgt atg aaa cag cct cct att    961
Gly Asp Pro Glu Ser Phe Lys Lys Phe Arg Met Lys Gln Pro Pro Ile
        305                 310                 315

gct tac tgg tta gaa act aaa aag gga agg aaa ggc tgg tta cag aaa    1009
Ala Tyr Trp Leu Glu Thr Lys Lys Gly Arg Lys Gly Trp Leu Gln Lys
320                 325                 330                 335

tat atg cct gct tta cct cat gga agc aaa tac agg gtg tat ttt aac    1057
Tyr Met Pro Ala Leu Pro His Gly Ser Lys Tyr Arg Val Tyr Phe Asn
                340                 345                 350

aca cca aat ggg cct ctt gaa cga gtt cct gcg tgg gcc aat ttt gtc    1105
Thr Pro Asn Gly Pro Leu Glu Arg Val Pro Ala Trp Ala Asn Phe Val
                355                 360                 365

att cca gat gca ggc ggg atg gca tta gca gtc cat tgg gaa cca cct    1153
Ile Pro Asp Ala Gly Gly Met Ala Leu Ala Val His Trp Glu Pro Pro
            370                 375                 380

cct gaa tat gct tat aaa tgg aaa cac aag cta cca gtc aag cct aag    1201
Pro Glu Tyr Ala Tyr Lys Trp Lys His Lys Leu Pro Val Lys Pro Lys
        385                 390                 395

tcc ttg cgc ata tat gaa tgt cat gtt ggc atc tct ggc cag gaa cca    1249
Ser Leu Arg Ile Tyr Glu Cys His Val Gly Ile Ser Gly Gln Glu Pro
400                 405                 410                 415

aaa gtt tca tct ttc aat gat ttt att agc aag gtc ctt ccg cat gta    1297
Lys Val Ser Ser Phe Asn Asp Phe Ile Ser Lys Val Leu Pro His Val
                420                 425                 430

aaa gaa gct gga tac aat gca ata caa att att gga gtt gtt gag cac    1345
Lys Glu Ala Gly Tyr Asn Ala Ile Gln Ile Ile Gly Val Val Glu His
                435                 440                 445

aag gat tat ttc act gtt gga tat aga gtg acc aat ttt tat gct gtt    1393
Lys Asp Tyr Phe Thr Val Gly Tyr Arg Val Thr Asn Phe Tyr Ala Val
            450                 455                 460

agt agc cgt tat ggc aca ccg gat gac ttc aag cgc ttg gtt gat gaa    1441
Ser Ser Arg Tyr Gly Thr Pro Asp Asp Phe Lys Arg Leu Val Asp Glu
        465                 470                 475

gca cat ggg ctt gga ctg ctt gtc ttt ttg gag att gtg cac tct tat    1489
Ala His Gly Leu Gly Leu Leu Val Phe Leu Glu Ile Val His Ser Tyr
480                 485                 490                 495

gca gca gca gat gaa atg gtt ggg tta tct ctt ttt gat gga gca aat    1537
Ala Ala Ala Asp Glu Met Val Gly Leu Ser Leu Phe Asp Gly Ala Asn
                500                 505                 510

gat tgc tat ttc cac act ggt aaa cgt gga cac cac aaa ttc tgg ggc    1585
Asp Cys Tyr Phe His Thr Gly Lys Arg Gly His His Lys Phe Trp Gly
            515                 520                 525
```

BCS 03-5004_SEQUENZPROTOKOLL.ST25

```
aca cgg atg ttc aaa tat gga gat ctt gat gtt ctg cac ttt ctt ctt      1633
Thr Arg Met Phe Lys Tyr Gly Asp Leu Asp Val Leu His Phe Leu Leu
        530             535             540

tca aat ctg aac tgg tgg gtg gag gag tat cat gtc gat ggc ttc cat      1681
Ser Asn Leu Asn Trp Trp Val Glu Glu Tyr His Val Asp Gly Phe His
    545             550             555

ttt cat tcg ctc tcg tcc atg ttg tat acg cat aat gga ttt gct tca      1729
Phe His Ser Leu Ser Ser Met Leu Tyr Thr His Asn Gly Phe Ala Ser
560             565             570             575

ttt act ggt gac atg gat gaa tac tgt aac caa tat gtt gac aag gag      1777
Phe Thr Gly Asp Met Asp Glu Tyr Cys Asn Gln Tyr Val Asp Lys Glu
                580             585             590

gcc tta ttg tac ctc ata tta gca aat gaa gta tta cat gct ctt cat      1825
Ala Leu Leu Tyr Leu Ile Leu Ala Asn Glu Val Leu His Ala Leu His
        595             600             605

cct aat gtg atc acg att gct gag gat gca act ctg tat cct gga ctc      1873
Pro Asn Val Ile Thr Ile Ala Glu Asp Ala Thr Leu Tyr Pro Gly Leu
        610             615             620

tgc gat cca aca tct caa ggt gga ctg ggc ttt gat tat ttt gcc aat      1921
Cys Asp Pro Thr Ser Gln Gly Gly Leu Gly Phe Asp Tyr Phe Ala Asn
    625             630             635

ctt tct gcc tca gag atg tgg ctt gca tta ctt gaa aat act cct gat      1969
Leu Ser Ala Ser Glu Met Trp Leu Ala Leu Leu Glu Asn Thr Pro Asp
640             645             650             655

cat gaa tgg tgc atg agt aag att gtt agc aca tta gtg ggc gat aga      2017
His Glu Trp Cys Met Ser Lys Ile Val Ser Thr Leu Val Gly Asp Arg
                660             665             670

caa aat act gat aaa atg ctt ttg tat gca gaa aat cac aac cag tcc      2065
Gln Asn Thr Asp Lys Met Leu Leu Tyr Ala Glu Asn His Asn Gln Ser
            675             680             685

att tct gga ggt cgt tcc ttc gca gaa ata ctg att ggt aac tcc ttg      2113
Ile Ser Gly Gly Arg Ser Phe Ala Glu Ile Leu Ile Gly Asn Ser Leu
        690             695             700

ggg aaa tcc tcc ata tca caa gag tca tta ctt aga ggc tgc tcg tta      2161
Gly Lys Ser Ser Ile Ser Gln Glu Ser Leu Leu Arg Gly Cys Ser Leu
    705             710             715

cac aag atg atc aga tta att aca tct aca att ggt ggt cat gca tac      2209
His Lys Met Ile Arg Leu Ile Thr Ser Thr Ile Gly Gly His Ala Tyr
720             725             730             735

ctc aac ttc atg ggc aat gaa ttt ggt cac cca aag aga gta gag ttt      2257
Leu Asn Phe Met Gly Asn Glu Phe Gly His Pro Lys Arg Val Glu Phe
                740             745             750

cca atg tca agc aac aat ttc tcc ttt tca ctg gct aac cgt cgc tgg      2305
Pro Met Ser Ser Asn Asn Phe Ser Phe Ser Leu Ala Asn Arg Arg Trp
            755             760             765

gat cta ttg gaa gat gtt gta cat tat caa ttg ttc tca ttt gat aag      2353
Asp Leu Leu Glu Asp Val Val His Tyr Gln Leu Phe Ser Phe Asp Lys
        770             775             780

ggt atg atg gac ttg gat aaa aat ggg aga att ttg tcc aga ggt ctt      2401
Gly Met Met Asp Leu Asp Lys Asn Gly Arg Ile Leu Ser Arg Gly Leu
        785             790             795
```

BCS 03-5004_SEQUENZPROTOKOLL.ST25

```
gcc aac att cac cat gtc aat gat act acc atg gtg att tct tac ttg   2449
Ala Asn Ile His His Val Asn Asp Thr Thr Met Val Ile Ser Tyr Leu
800                   805                 810                 815

aga ggt ccc aat ctc ttt gtg ttc aac ttt cat cct gtc aat tca tat   2497
Arg Gly Pro Asn Leu Phe Val Phe Asn Phe His Pro Val Asn Ser Tyr
                  820                 825                 830

gaa aga tac att ata ggt gtg gaa gaa gct gga gag tat caa gtc aca   2545
Glu Arg Tyr Ile Ile Gly Val Glu Glu Ala Gly Glu Tyr Gln Val Thr
              835                 840                 845

tta aat aca gat gaa aac aag tat ggt ggt aga gga cta ctt ggc cat   2593
Leu Asn Thr Asp Glu Asn Lys Tyr Gly Gly Arg Gly Leu Leu Gly His
          850                 855                 860

gat cag aat att caa aga acc att agt aga aga gct gat gga atg aga   2641
Asp Gln Asn Ile Gln Arg Thr Ile Ser Arg Arg Ala Asp Gly Met Arg
      865                 870                 875

ttt tgc ttg gaa gtg cct ctg cca agt aga agt gct cag gtc tac aag   2689
Phe Cys Leu Glu Val Pro Leu Pro Ser Arg Ser Ala Gln Val Tyr Lys
880                 885                 890                 895

ttg acc cga att cta aga gca tgatcactct agtaatcaaa gtgcctcata       2740
Leu Thr Arg Ile Leu Arg Ala
                  900

tgatgacaca aaaggaaagg ttctacattg cccttacact gatcaatatt gacacctttc  2800

cgaggtgagt ttctgtgatt cttgagcaga ctgttggcta gtcaattatc atgaactttt  2860

gccttcagca tccggatagt cgcttctcct gtgcaatgag ggcatggacg aatttttttt  2920

tggcttgtca tggggggtcat aagcatccgc cagattaaga tttcacaggc ctcgagtaaa  2980

accatcactt actttaagga tacacaaaca caccaacggg gtgcaggctc tgataccttc  3040

taaagtg                                                            3047
```

<210> 6

<211> 902

<212> PRT

<213> Solanum tuberosum

<400> 6

```
Met Leu Ser Leu Ser Asp Ser Ile Arg Ile Ser Ser Pro Leu Ser Asp
1               5                   10                  15

Ser Arg Leu Ser Phe Leu Ser Gln Thr Gly Ser Arg Thr Ser Arg Gln
              20                  25                  30

Leu Lys Phe Val Arg Ser Arg Arg Ala Arg Val Ser Arg Cys Arg Cys
          35                  40                  45

Ser Ala Thr Glu Gln Pro Pro Pro Gln Arg Arg Lys Gln Arg Pro Glu
      50                  55                  60
```

BCS 03-5004_SEQUENZPROTOKOLL.ST25

Lys Tyr Lys Gln Ser Glu Glu Gly Lys Gly Ile Asp Pro Val Gly Phe
65   70   75   80

Leu Ser Lys Tyr Gly Ile Thr His Lys Ala Phe Ala Gln Phe Leu Arg
85   90   95

Glu Arg Tyr Lys Ser Leu Lys Asp Leu Lys Asp Glu Ile Leu Thr Arg
100   105   110

His Phe Ser Leu Lys Glu Met Ser Thr Gly Tyr Glu Leu Met Gly Met
115   120   125

His Arg Asn Ile Gln His Arg Val Asp Phe Leu Glu Trp Ala Pro Gly
130   135   140

Ala Arg Tyr Cys Ala Leu Ile Gly Asp Phe Asn Gly Trp Ser Thr Thr
145   150   155   160

Gly Asn Cys Ala Arg Glu Gly His Phe Gly His Asp Asp Tyr Gly Tyr
165   170   175

Trp Phe Ile Ile Leu Glu Asp Lys Leu Arg Glu Gly Glu Glu Pro Asp
180   185   190

Lys Leu Tyr Phe Gln Gln Tyr Asn Tyr Ala Glu Asp Tyr Gly Lys Gly
195   200   205

Asp Thr Gly Ile Thr Val Glu Glu Ile Phe Lys Lys Ala Asn Asp Glu
210   215   220

Tyr Trp Glu Pro Gly Glu Asp Arg Phe Ile Lys Ser Arg Tyr Glu Val
225   230   235   240

Ala Ala Lys Leu Tyr Glu Glu Met Phe Gly Pro Asn Gly Pro Gln Thr
245   250   255

Glu Glu Glu Leu Glu Ala Met Pro Asp Ala Ala Thr Arg Tyr Lys Thr
260   265   270

Trp Lys Glu Gln Gln Lys Glu Asp Pro Ala Ser Asn Leu Pro Ser Tyr
275   280   285

Asp Val Val Asp Ser Gly Lys Glu Tyr Asp Ile Tyr Asn Ile Ile Gly
290   295   300

Asp Pro Glu Ser Phe Lys Lys Phe Arg Met Lys Gln Pro Pro Ile Ala
305   310   315   320

Tyr Trp Leu Glu Thr Lys Lys Gly Arg Lys Gly Trp Leu Gln Lys Tyr
325   330   335

BCS 03-5004_SEQUENZPROTOKOLL.ST25

```
Met Pro Ala Leu Pro His Gly Ser Lys Tyr Arg Val Tyr Phe Asn Thr
        340             345             350

Pro Asn Gly Pro Leu Glu Arg Val Pro Ala Trp Ala Asn Phe Val Ile
        355             360             365

Pro Asp Ala Gly Gly Met Ala Leu Ala Val His Trp Glu Pro Pro Pro
        370             375             380

Glu Tyr Ala Tyr Lys Trp Lys His Lys Leu Pro Val Lys Pro Lys Ser
385             390             395             400

Leu Arg Ile Tyr Glu Cys His Val Gly Ile Ser Gly Gln Glu Pro Lys
            405             410             415

Val Ser Ser Phe Asn Asp Phe Ile Ser Lys Val Leu Pro His Val Lys
            420             425             430

Glu Ala Gly Tyr Asn Ala Ile Gln Ile Ile Gly Val Val Glu His Lys
        435             440             445

Asp Tyr Phe Thr Val Gly Tyr Arg Val Thr Asn Phe Tyr Ala Val Ser
    450             455             460

Ser Arg Tyr Gly Thr Pro Asp Asp Phe Lys Arg Leu Val Asp Glu Ala
465             470             475             480

His Gly Leu Gly Leu Leu Val Phe Leu Glu Ile Val His Ser Tyr Ala
            485             490             495

Ala Ala Asp Glu Met Val Gly Leu Ser Leu Phe Asp Gly Ala Asn Asp
        500             505             510

Cys Tyr Phe His Thr Gly Lys Arg Gly His His Lys Phe Trp Gly Thr
        515             520             525

Arg Met Phe Lys Tyr Gly Asp Leu Asp Val Leu His Phe Leu Leu Ser
    530             535             540

Asn Leu Asn Trp Trp Val Glu Glu Tyr His Val Asp Gly Phe His Phe
545             550             555             560

His Ser Leu Ser Ser Met Leu Tyr Thr His Asn Gly Phe Ala Ser Phe
            565             570             575

Thr Gly Asp Met Asp Glu Tyr Cys Asn Gln Tyr Val Asp Lys Glu Ala
        580             585             590

Leu Leu Tyr Leu Ile Leu Ala Asn Glu Val Leu His Ala Leu His Pro
        595             600             605
```

Seite 16

BCS 03-5004_SEQUENZPROTOKOLL.ST25

```
Asn Val Ile Thr Ile Ala Glu Asp Ala Thr Leu Tyr Pro Gly Leu Cys
    610             615             620
```

```
Asp Pro Thr Ser Gln Gly Gly Leu Gly Phe Asp Tyr Phe Ala Asn Leu
625             630             635             640
```

```
Ser Ala Ser Glu Met Trp Leu Ala Leu Leu Glu Asn Thr Pro Asp His
            645             650             655
```

```
Glu Trp Cys Met Ser Lys Ile Val Ser Thr Leu Val Gly Asp Arg Gln
        660             665             670
```

```
Asn Thr Asp Lys Met Leu Leu Tyr Ala Glu Asn His Asn Gln Ser Ile
        675             680             685
```

```
Ser Gly Gly Arg Ser Phe Ala Glu Ile Leu Ile Gly Asn Ser Leu Gly
    690             695             700
```

```
Lys Ser Ser Ile Ser Gln Glu Ser Leu Leu Arg Gly Cys Ser Leu His
705             710             715             720
```

```
Lys Met Ile Arg Leu Ile Thr Ser Thr Ile Gly Gly His Ala Tyr Leu
            725             730             735
```

```
Asn Phe Met Gly Asn Glu Phe Gly His Pro Lys Arg Val Glu Phe Pro
            740             745             750
```

```
Met Ser Ser Asn Asn Phe Ser Phe Ser Leu Ala Asn Arg Arg Trp Asp
    755             760             765
```

```
Leu Leu Glu Asp Val Val His Tyr Gln Leu Phe Ser Phe Asp Lys Gly
    770             775             780
```

```
Met Met Asp Leu Asp Lys Asn Gly Arg Ile Leu Ser Arg Gly Leu Ala
785             790             795             800
```

```
Asn Ile His His Val Asn Asp Thr Thr Met Val Ile Ser Tyr Leu Arg
            805             810             815
```

```
Gly Pro Asn Leu Phe Val Phe Asn Phe His Pro Val Asn Ser Tyr Glu
        820             825             830
```

```
Arg Tyr Ile Ile Gly Val Glu Glu Ala Gly Glu Tyr Gln Val Thr Leu
        835             840             845
```

```
Asn Thr Asp Glu Asn Lys Tyr Gly Gly Arg Gly Leu Leu Gly His Asp
    850             855             860
```

```
Gln Asn Ile Gln Arg Thr Ile Ser Arg Arg Ala Asp Gly Met Arg Phe
865             870             875             880
```

BCS 03-5004_SEQUENZPROTOKOLL.ST25

Cys Leu Glu Val Pro Leu Pro Ser Arg Ser Ala Gln Val Tyr Lys Leu
            885   890   895

Thr Arg Ile Leu Arg Ala
            900

SwissProt Acc No. or Entry Name	Amino acid No.	SwissProt Acc No. or Entry Name	Amino acid No.
APU_THETU	1251-1331	Q9XED2	101-191
GLGB_SYNY3	22-110	Q08131	137-227
P71095	39-130	GLGB_HUMAN	73-168
Q9RXB0	181-274	Q9V6K7	52-144
PULA_KLEPN	301-395	Q22137	53-147
P70983	1143-1238	Q9RM63	25-149
Q41386	205-298	ISOA_FLASP	36-163
O64454	202-295	ISOA_PSEAY	30-155
O69008	105-191	P73608	22-122
O34587	104-189	O04196	74-177
Q9XDB5	231-319	Q9SPT7	8-110
PULA_THEMA	223-311	Q41742	114-218
Q59319	206-300	GLGX_HAEIN	10-101
YIEL_ECOLI	41-123	Q9RNH5	20-117
Q9RX51	22-100	GLGX_ECOLI	9-104
O66936	24-116	Q9RXP5	13-108
Q59832	141-233	GLGX_MYCTU	24-119
GLGB_STRAU	160-252	Q9X947	18-113
GLGB_BUTFI	24-116	P72691	19-120
GLGB_AGRTU	130-223	P95868	17-117
Q9RQI5	134-226	O84046	11-108
GLGB_ECOLI	122-214	Q9Z8F5	11-108
GLGB_HAEIN	122-214	Q9ZVT2	232-335
GLGB_SYNY3	126-217	Q44528	2-83
GLGB_MYCTU	127-223	Q9X2G0	276-357
Q9RTB7	26-115	Q9X2G0	15-98
GLGB_BACSU	23-115	Q45643	46-114
Q59242	23-115	Q9X2G0	126-217
O84874	117-209	TREZ_ARTSQ	5-90
O49185	62-153	TREZ_MYCTU	2-68
GLGB_YEAST	59-153	Q55088	1-79
Q9Y8H3	47-147	Q53641	1-79

Table 1   Amino acid sequences, contained in the "seed alignment", that are used for producing the HMM for the Pfam isoamylase domain (PF 02922). The table gives the "accession" number (Acc No) or the name (Entry Name), under which the corresponding amino acid sequences are entered in the SwissProt database. Those sections of the amino acid sequences of the corresponding SwissProt entry, which are part of the "seed alignment" (amino acid No.), are also given.

SwissProt Acc No. or Entry Name	Amino acid No.	SwissProt Acc No. or Entry Name	Amino acid No.
AMYM_BACLI	137-479	CDG1_PAEMA	46-426
MALZ_ECOLI	128-522	CDGT_BACOH	44-420
APU_THESA	393-821	AMYB_PAEPO	751-1107
APU_THEET	390-820	AMYA_ASPOR	34-390
CDAS_THEET	136-494	AMY1_DEBOC	49-405
NEPU_BACST	139-497	AMY1_SACFI	40-396
AMYM_BACAD	139-497	AMY1_ECOLI	193-611
AMY2_DICTH	138-470	ISOA_PSEAY	209-652
MALT_AEDAE	29-425	AMY_BUTFI	126-520
MAL2_DROME	30-432	AMY_BACSU	41-383
MAL3_DROME	31-428	AMY_THECU	40-392
MAL1_DROME	35-420	AMY_STRHY	37-360
MAXS_YEAST	17-441	AMY_STRGR	35-372
MAYS_YEAST	22-446	AMY_ALTHA	28-373
MA3S_YEAST	21-443	AMYA_AERHY	26-369
TREC_ECOLI	15-414	AMYC_HUMAN	26-413
TREC_BACSU	16-418	AMYA_DROME	29-396
O16G_BACSP	11-420	AMY1_AERHY	22-379
O16G_BACCE	13-419	AMT4_PSESA	38-387
DEXB_STRMU	13-394	AMY2_ECOLI	12-402
AMY_BACME	44-406	AMY_BACLI	34-420
AMY3_DICTH	39-381	AMY_BACAM	34-422
AMY_STRLI	77-520	AMT6_BACS7	40-426
CDGT_KLEPN	47-463	AMY3_WHEAT	26-348
AMYM_BACST	46-430	AM3A_ORYSA	29-367
CDGT_BACST	47-425	AMYA_VIGMU	24-361
AMYR_BACS8	46-425	AM2A_ORYSA	23-366

Table 2   Amino acid sequences, contained in the "seed alignment", that are used for producing the HMM for the Pfam alpha-amylase domain (PF 00128). The table gives the "accession" number (Acc No) or the name (Entry Name), under which the corresponding amino acid sequences are entered in the SwissProt database. Those sections of the amino acid sequences of the corresponding SwissProt entry, which are part of the "seed alignment" (amino acid No.), are also given.

Table 3 Information for producing the HMM for the Pfam isoamylase domain (PF 02922)

```
HMMER  R2.0 [2.3.1]
NAME   isoamylase_N
ACC    PF02922
DESC   Isoamylase N-term
LENG   121
ALPH   Amino
RF     no
CS     no
MAP    yes
COM    hmmbuild -F HMM_is.ann SEED.ann
COM    hmmcalibrate --seed 0 HMM_is.ann
NSEQ   65
DATE
CKSUM  3012
GA     2.3 2.3
TC     2.3 2.3
NC     2.1 2.1
XT     -8455    -4  -8455    -4  -1000  -1000  -8455    -4  -8455    -4
NULT      -4  -8455
NULE     595  -1558    85   338  -294   453  -1158   197   249   902  -1085  -142   -21  -313    45   531   201   384  -1998  -644
EVD   -56.839790 0.222419
```

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	
	m->m	m->i	m->d	i->m	i->i	d->m	d->d	b->m	m->e												
	-32	*	-5527																		
1	433	-2912	-5203	-4583	312	-4575	-3427	-2412	-4218	-2763	739	-4157	2303	-3877	-1058	-777	159	-2330	1852	3373	1
.	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
.	-1	-10660	-11703	-894	-1115	-701	-1378	*	*												
2	-503	-4276	-455	1534	635	1339	-2632	-1609	373	78	463	-1115	-680	241	-2737	-924	-593	-1619	43	316	2
.	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
.	-1	-10857	-11899	-894	-1115	-701	-1378														
3	-806	-4386	-2899	-88	-1199	-4001	-313	-1878	1255	-1032	-3488	-2653	2668	-172	105	545	387	-400	-4594	-3936	3
.	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
.	-1	-10916	-11958	-894	-1115	-701	-1378														
4	-1972	-3101	-5615	-1806	2143	-2106	-3892	-829	-4575	2525	1112	-4465	-4871	-4199	-4376	-1753	-1159	-2514	-3558	1139	4
.	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
.	-1	-10916	-11958	-894	-1115	-701	-1378														
5	-1028	-6327	-1172	-753	-8643	3551	-4321	-471	-1174	-6434	-5732	-3689	-5515	-3996	-5049	-4652	-4943	-6008	166	-5877	5
.	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
.	-1	-10941	-11983	-894	-1115	-701	-1378														
6	2712	446	-5632	-1558	482	-1817	-3713	-635	-4593	-29	-2327	-4485	109	-4218	-4397	-260	-3237	343	-3582	161	6
.	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
.	-1	-10941	-11983	-894	-1115	-701	-1378														

Table 3, Sheet 1/9

Table 3, Sheet 2/9

Matrix block (rows 7–20; each cell shown as "value / column‑header value"):

Row	–249	–294	–369	117	359	96	45	394	275	–720	–466	210	–626
7	–3981	–4653	312	1327	428	–67	–102	–4102	1206	–169	–4467 (–468)	–1	–580
8	2394	1850	834	–1672	–1606	168	–1158	1637	–4198	–2400	–603	–159	346
9	380	–4365	–577	–340	144	–268	1174	–1346	244	–3267	322	–1953	7
10	–15	683	–1189	–514	164	99	–1590	1626	–2026	–2963	–1843	–458	–3935
11	–3345	–4026	–888	–855	–2257	–2097	775	230	448	–2931	–1532	467	–1276
12	–2959	–3637	–3067	912	–621	–253	738	–3064	1275	2049	–1423	389	–810
13	–3831	–4646	–4063	2401	–2580	–2778	823	–3560	1207	–3642	–4468	–2156	–4550
14	–1529	–1876	1517	–1503	–2182	–2591	–2380	–3139	–2883	1929	–141	–2718	1265
15	–2315	–2998	–289	–1284	–1224	–1068	683	–2408	1507	–1903	–2828	499	–2880
16	–5237	–5331	335	–2415	816	–4699	–4449	477	–3556	–4214	–5141	–4947	–4858
17	–2410	–3046	802	–1440	–1401	–1233	–729	1166	–1122	–1944	–2816	864	–2783
18	–3260	–3933	2044	459	–2198	–2044	–1495	–3378	353	–2833	–1513	1272	–3779
19	–266	–4172	–1409	–470	203	–585	395	–1034	1770	–3078	–4005	805	–4060
20	–771	–4659	–4098	–490	81	–434	188	–360	–12	–3565	–4492	627	–696

Second matrix block (rows 7–20; column‑header values 106, 399, –381, 43, 233, –500, –149):

Row	106	399	–381	43	233	–500	–149
7	3316	–4010	–1229	183	–2893	–4458	–1172
8	–3587	–4749	1372	–841	–5058	–3201	–427
9	276	–1316	–4483	593	1665	121	–554
10	–2048	–3623	–4188	858	–2164	–10608	–365
11	–2009	–4637	–810	1314	616	–3873	797
12	1139	–1364	–3762	–1300	1826	–10244	–46
13	–2276	892	–4735	2261	1648	–3841	–14
14	1869	–398	–1379	1196	–1367	–10201	–1075
15	1306	–5563	602	288	–2404	832	–847
16	–4399	–240	–5091	–5046	–3700	–9739	–511
17	–1158	3282	–3091	2128	2298	–4407	–2797
18	–1948	–2384	–4041	–454	–3483	–9232	–305
19	–2148	–2427	–4310	2	–4788	1322	–88
20	–198	–89	–1213	–506	–898	–8931	–149

(Additional header/boundary values associated with row 7: –1378, –701, –1115, –894, –11983, –10941, –1; large diagonal values appearing in the –500 column include –11983, –10941, –9845, –11137, –10365, –10917.)

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1	512 / -249	444 / -249	1564 / -249	531 / -249	265 / -249	2570 / -249	-5256 / -249	-4985 / -249	700 / -249	-5893 / -249	-834 / -249	-868 / -249	-4988 / -249	910 / -249	-5807 / -249
2	1631 / -294	-4694 / -294	-3659 / -294	-4578 / -294	-3750 / -294	5535 / -294	392 / -294	-5617 / -294	-4755 / -294	-6500 / -294	-4739 / -294	122 / -294	-5365 / -294	-4745 / -294	-5892 / -294
3	2518 / -369	714 / -369	-410 / -369	-3897 / -369	2623 / -369	-6697 / -369	-4553 / -369	-4961 / -369	-682 / -369	-1832 / -369	-788 / -369	-1807 / -369	3460 / -369	-1017 / -369	2356 / -369
4	2145 / 117	1578 / 117	-3321 / 117	896 / 117	-3396 / 117	-7488 / 117	97 / 117	-1675 / 117	1414 / 117	-4321 / 117	1202 / 117	-638 / 117	-4511 / 117	976 / 117	-5011 / 117
5	-4006 / 359	-1028 / 359	-1974 / 359	501 / 359	-4099 / 359	-7150 / 359	2037 / 359	697 / 359	-822 / 359	178 / 359	-590 / 359	470 / 359	-5634 / 359	1507 / 359	-6367 / 359
6	-4478 / 96	1656 / 96	-1411 / 98	2409 / 96	-4568 / 96	-1618 / 96	-4834 / 96	-1215 / 96	-1480 / 96	-4921 / 96	1235 / 96	1510 / 96	-2134 / 96	962 / 96	-6803 / 96
7	-4301 / 45	-2312 / 45	-4306 / 45	-2403 / 45	-4386 / 45	-6639 / 45	-4560 / 45	-3175 / 45	261 / 45	-1072 / 45	1892 / 45	-33 / 45	-5948 / 45	-413 / 45	-6529 / 45
8	-543 / 394	-4189 / 394	-4977 / 394	-1077 / 394	-5058 / 394	-7759 / 394	-5320 / 394	3730 / 394	-4166 / 394	-811 / 394	-4151 / 394	-1906 / 394	-6412 / 394	-4169 / 394	-6949 / 394
9	-4567 / 275	1974 / 275	-4572 / 275	-2834 / 275	-4654 / 275	-6513 / 275	-1206 / 275	-3477 / 275	2138 / 275	-1004 / 275	-983 / 275	1027 / 275	-6118 / 275	-2714 / 275	-6804 / 275
10	-2403 / -720	-3587 / -720	-2409 / -720	-3447 / -720	-2465 / -720	-5949 / -720	-4458 / -720	-4564 / -720	-3661 / -720	-5432 / -720	-883 / -720	-3661 / -720	-433 / -720	-3649 / -720	-3458 / -720
11	-1338 / -466	-4488 / -466	-325 / -466	-2327 / -466	1669 / -466	-5859 / -466	-1991 / -466	-5465 / -466	-4587 / -466	-6303 / -466	-2673 / -466	-2337 / -466	-1110 / -466	-2183 / -466	2467 / -466
12	-4678 / 210	-450 / 210	-4684 / 210	963 / 210	-4761 / 210	-7815 / 210	-1677 / 210	-1616 / 210	-74 / 210	-4423 / 210	1147 / 210	352 / 210	-6240 / 210	587 / 210	-6901 / 210
13	860 / -626	408 / -626	-432 / -626	-1804 / -626	-1194 / -626	-6546 / -626	-4952 / -626	-5508 / -626	-2129 / -626	-6257 / -626	-993 / -626	-4643 / -626	-826 / -626	-1791 / -626	540 / -626
14	-3792 / 106 / -1378	-526 / 106 / -1378	-3796 / -106 / -1378	-2821 / 106 / -1378	-3887 / 106 / -1378	-4102 / 106 / -1378	-4712 / 106 / -1378	-3603 / 106 / -1378	2269 / 106 / -1378	-4534 / 106 / -1378	-2716 / 106 / -1916	-470 / 106 / -1378	-5600 / 106 / -1378	-481 / 106 / -1378	-6387 / 106 / -1378
15	-1893 / 399 / -701	-4097 / 399 / -701	-2056 / 399 / -701	-4149 / 399 / -701	-5013 / 399 / -701	-1893 / 399 / -701	-4813 / 399 / -701	-1752 / 399 / -701	336 / 399 / -701	-2136 / 399 / -701	-4058 / 444 / -701	489 / 399 / -701	-8466 / 399 / -701	-4076 / 399 / -701	-1604 / 399 / -701
16	-433 / -381 / -1115	190 / -381 / -1115	3969 / -381 / -1115	-4550 / -381 / -1115	-116 / -381 / -1115	2064 / -381 / -1115	-5332 / -381 / -1115	-5759 / -381 / -1115	-4892 / -381 / -1115	-6477 / -381 / -1115	-4875 / -381 / -1115	-1179 / -381 / -1115	-4477 / -381 / -1115	-499 / -381 / -1115	-1252 / -381 / -1115
17	-5083 / 43 / -894	-132 / 43 / -894	-5089 / 43 / -894	-2546 / 43 / -894	931 / 43 / -894	-1924 / 43 / -894	-4910 / 43 / -894	156 / 43 / -894	163 / 43 / -894	-1527 / 43 / -894	1463 / 43 / -894	954 / 43 / -894	-6525 / 43 / -894	758 / 43 / -894	-7120 / 43 / -894
18	-5719 / 233 / -12065	-1380 / 233 / -12065	-5724 / 233 / -12065	-1346 / 233 / -12065	-1253 / 233 / -12065	-7990 / 233 / -12065	-5323 / 233 / -12065	-3578 / 233 / -12065	-945 / 233 / -12065	-3960 / 233 / -12065	-6420 / 148 / -12048	-491 / 233 / -12065	-7042 / 233 / -12065	-156 / 233 / -12065	-7592 / 233 / -12065
19	-3200 / -500 / -11023	-4488 / -500 / -11023	-3207 / -500 / -11023	1196 / -500 / -11023	-3277 / -500 / -11023	-6573 / -500 / -11023	-4354 / -500 / -11023	-4943 / -500 / -11023	-4571 / -500 / -11023	-5060 / -500 / -11023	-4555 / -500 / -11023	-4572 / -500 / -11023	-7042 / -500 / -11023	-4558 / -500 / -11023	-4635 / -500 / -11023
20	-903 / -149 / -1	-1902 / -149 / -1	-1835 / -149 / -1	1377 / -149 / -1	-1482 / -149 / -1	-7635 / -149 / -1	2849 / -149 / -1	-464 / -149 / -1	-36 / -149 / -1	3362 / -149 / -1	-1049 / -18 / -1	331 / -149 / -1	922 / -149 / -1	619 / -149 / -1	-2161 / -149 / -1

Table 3, Sheet 3/9

	36	37	38	39	40	41	42	43	44	46	47	48	49	50
	-218 -249	-3925 -249	2201 -249	-4072 -249	-4075 -249	96 -249	-263 -249	-2869 -249	-2292 -246	-2736 -249	-3708 -249	381 -249	50 -249	-4067 -249
	-3666 -294	-4259 -294	-4386 -294	-4755 -294	-4758 -294	189 -294	-3757 -294	-3552 -294	-2974 -295	-3646 -294	5255 -294	-4303 -294	1382 -294	-4746 -294
	1652 -369	945 -369	-1060 -369	-1613 -369	-4198 -369	-678 -369	-3195 -369	-979 -369	-338 -370	-3025 -369	-1471 -369	495 -369	-242 -369	-488 -369
	194 117	-1925 117	-667 117	-637 117	-302 117	-459 117	735 117	-45 117	269 117	-1715 117	-905 117	-2712 117	819 117	807 117
	-1302 359	-4605 359	914 359	55 359	-914 359	-398 359	504 359	-1778 359	844 359	-1382 359	-2825 359	-137 359	-1260 359	22 359
	-1396 96	-5089 96	-3095 96	-1098 96	-1400 86	-215 96	-1822 98	-284 96	447 95	-1954 96	845 96	-2548 96	-654 98	1235 98
	-1129 45	-4899 45	-1254 45	-2272 45	-2273 45	-772 45	-1275 45	1860 45	-494 48	-734 45	-385 45	383 45	159 45	1003 45
	-4956 394	-5523 394	-198 394	-4166 394	1201 394	194 394	-267 394	-226 394	64 393	2482 394	-1213 394	980 394	461 394	-4169 394
	-4518 275	-5161 275	-282 275	-715 275	3036 275	2273 275	1007 275	771 275	897 275	1962 275	-2355 275	1492 275	-2734 275	536 275
	-41 -720	-2773 -720	-3222 -720	-3660 -720	-3684 -720	-3593 -720	-2663 -720	-2457 -720	-1880 -721	-2733 -720	-3291 -720	-3186 -720	-3612 -720	1409 -720
	248 -466	1969 -466	-1377 -466	-4587 -466	-1673 -466	-2300 -486	-1313 -466	-100 -466	-2805 -466	-3463 -466	-1027 -466	-2023 -466	-1569 -466	-1440 -466
	140 210	-5277 210	-2644 210	414 210	367 210	-2246 210	-50 210	-1111 210	1750 210	-1203 210	689 210	-2051 210	-98 210	-725 210
	1189 -626	-501 -626	-1027 -628	-4642 -626	-4646 -626	-4574 -626	-3645 -626	-3438 -626	-2859 -627	-3545 -626	-4263 -626	-815 -626	-242 -626	-119 -626
	-248 106 -1378	-4436 106 -1378	-2937 106 -1378	570 106 -1378	-2733 106 -1378	1065 108 -337	-1734 108	-40 106	-953 105 -28	-1077 106 -2026	303 106 -185	1342 106 -4726	465 106 -1378	885 108 -1378
	-4905 399 -701	2370 399 -701	-919 399 -701	-452 399 -701	-1946 399 -701	1166 399	-2265 399	-5207	1508 399 -5379	845 398 -5694	-1761 399 -407	-3717 399 -3058	-3775 398 -56	1230 399 -701	-908 399 -701
	-388 -381 -1115	-56 -381 -1115	1807 -381 -1115	3174 -381 -1115	-4895 -381 -1115	-4824 -381 -1115	-3894 -381 -1115	-3688 -381 -1115	-3110 -381 -4913	-3659 -381 -1115	-4517 -381 -1115	-880 -381 -1115	-1260 -381 -1115	96 -381 -1115
	-4985 43 -894	-5655 43 -894	-411 43 -894	-44 43 -894	369 43 -894	142 43 -894	899 43 -894	558 43 -894	-620 43 -49	-36 43 -894	509 43 -894	-120 43 -894	731 43 -894	1388 43 -894
	-1721 233 -12065	-6262 233	2121 233	1686 233	362 233	1404 233	1431 233	980 233	642 232 -638	2437 233	-915 233	2253 233	-1114 233	-1116 233 -12065
	2801 -500 -11023	-3659 -500	-4080 -500	-4571 -500	-4574 -500	-4504 -500	-3574 -500	-8872 -500	-2790 -500	-3285 -500	-4200 -500	-4079 -500	-4516 -500	-4560 -500 -11023
	557 -149 -1	-924 -149 -1	-3164 -149 -1	-1317 -149 -1	584 -149 -74	-1777 -149 -1080	10 -149 -258	-1897 -149 -786	-1320 -145 -4000	-1645 -149 -624	-2743 -149 -1	-1155 -149 -1	656 -149 -1	-3100 -149 -1

Table 3, Sheet 4/9

	51	52	53	54	55	56	57	58	59	60	61	62	63	64
	-4062 -249	285 -249	-4074 -249	798 -249	34 -249	183 -249	-2486 -249	1915 -249	-1370 -249	-3413 -249	-3676 -249	-3773 -249	-209 -249	421 -249
	-4739 -294	-3814 -294	-4757 -294	-3668 -294	-4755 -294	-4347 -294	-3108 -294	-2676 -294	-1816 -294	-4095 -294	-4359 -294	-4459 -294	-4687 -294	-4953 -294
	-254 -369	1148 -369	-260 -369	-593 -369	260 -369	-1625 -369	-2441 -369	-2134 -369	886 -369	479 -369	-978 -369	-3897 -369	-4126 -369	-4391 -369
	1279 117	41 117	-73 117	-3320 117	1286 117	-1052 117	714 117	-1082 117	1429 117	-2380 117	-1303 117	1898 117	-837 117	-3684 117
	554 359	-3740 359	-833 359	-4004 359	-880 359	493 359	1536 359	-1002 359	-1199 359	-1067 359	165 359	-881 359	636 359	-3660 359
	930 96	-67 96	619 96	-4465 96	903 96	1913 96	930 96	-717 96	1512 96	1057 96	1682 96	-304 96	-3662 96	-3662 96
	-814 45	-722 45	1442 45	-4279 45	-40 45	-1943 45	-894 45	-257 45	-782 45	-1615 45	-802 45	2 45	-1258 45	
	-4171 394	-4775 394	2995 394	-4971 394	59 394	1315 394	-2738 394	-2097 394	-2299 394	366 394	-3770 394	-329 394	-4754 394	
	-1344 275	364 275	-338 275	-4550 275	-2708 275	-768 275	-1326 275	-560 275	-1180 275	1723 275	976 275	1620 275	1368 275	
	698 -720	-2575 -720	-3663 -720	4259 -720	-3661 -720	-372 -720	-1982 -720	-1648 -720	1503 -720	-3000 -720	-3285 -720	-3593 -720	-746 -720	
	-2538 -466	332 -466	-4589 -466	1632 -466	-4587 -466	-594 -466	713 -466	-2508 -466	-66 -466	-227 -466	-577 -466	-4520 -466	-4786 -466	
	-1301 210	-703 210	493 210	-1610 210	1364 210	1748 210	201 210	1066 210	-885 210	1236 210	391 210	-1074 210	-3155 210	
	-824 -626	1305 -626	-4645 -626	-2702 -626	-1841 -626	-4166 -626	-330 -626	-2535 -626	-1010 -626	-3980 -626	-923 -626	-4575 -626	-4688 -626	
	-2738 106 -1378	3032 106 -1378	-2732 106 -1378	-3789 106 -1378	1453 106 -1378	392 106 -86	-1317 106 -69	-678 106 -184	-963 106 -1100	1889 106 -817	-235 106 -456	-2427 106 -2136	-313 106 -2966	-502 106 -1378

5 0	-1149 399 -701	-4712 399 -701	-4073 399 -701	-4921 399 -701	-1716 399 -701	-3731 399 -4109	918 399 -4411	1477 399 -3061	-2223 399 -906	-1517 399 -1209	825 399 -1882	133 399 -373	1699 399 -198	3185 399 -701
5 1	-4860 -381 -1115	439 -381 -1115	-4895 -381 -1115	-243 -381 -1115	-4892 -381 -1115	-415 -381 -1115	-3099 -381 -1115	-2771 -381 -1115	-1461 -381 -1115	-610 -381 -1115	-4496 -381 -1115	-4595 -381 -1115	1410 -381 -1115	-707 -381 -1115
5 2	1773 43 -894	-4044 43 -894	669 43 -894	-1804 43 -894	1153 43 -894	504 43 -894	-1035 43 -894	1978 43 -894	-1034 43 -894	615 43 -894	-249 43 -894	1673 43 -894	-81 43 -894	-2956 43 -894
5 3	-708 233 -12065	-1879 233	-58 233 -12065	-1623 233	654 233	-2038 233	129 233	-524 233	1440 233	36 233	-4270 233	2377 233	216 233	403 233 -12065
5 4	-115 -500 -11023	-3382 -500 -11023	-4573 -500 -11023	-3213 -500 -11023	-4571 -500 -11023	-4145 -500 -11023	-2867 -500 -9243	-2513 -500 -7810	-1426 -500 -8190	-3911 -500 -10278	-4175 -500 -10580	-4275 -500 -10626	-4504 -500 -10947	-4838 -500 -11023
5 5	941 -149 -1	-420 -149 -1	-1952 -149 -1	-1019 -149 -1	-1227 -149 -404	-2745 -149 -1381	-1634 -149 -1723	-1133 -149 -1253	-994 -149 -7	-2441 -149 -78	-272 -149 -81	-713 -149 -1	-443 -149 -1	-1102 -149 -1

Table 3, Sheet 5/9

Table 3, Sheet 6/9

	82	83	84	85	89	90	91	92	93	94	95	96	97	98	99
	-3580 -249	-3359 -249	-139 -249	-2661 -250	-2771 -249	1485 -249	1812 -249	4584 -249	5 -249	4039 -249	53 -249	236 -249	-1166 -249	-4011 -249	1464 -249
	-4253 -294	-4032 -294	-3635 -294	-2967 -295	-3453 -294	1903 -294	-4716 -294	-4919 -294	-4736 -294	261 -294	-4076 -294	-3938 -294	2043 -294	-4684 -294	9 -294
	-571 -369	-3452 -369	-3068 -369	-2556 -370	-2887 -369	1180 -369	-490 -369	209 -369	-1911 -369	-847 -369	-273 -369	2082 -369	658 -369	872 -369	-1085 -369
	-2565 117	-928 117	1572 117	-1559 120	-253 117	229 117	-545 117	-4829 117	-525 117	-3857 117	-1632 117	-3580 117	1115 117	898 117	-235 117
	-2514 359	-830 359	1907 359	-1448 358	-1682 359	-1179 359	-541 359	-5905 359	-1539 359	-4518 359	-2040 359	-4296 358	-1669 359	-592 358	-2982 359
	-2358 96	-450 96	-1710 96	-760 95	112 96	-3398 96	1884 96	-6337 96	-337 96	-4969 96	2679 96	-4765 96	1 96	342 96	-89 96
	-1812 45	-1588 45	1533 45	-964 44	490 45	1816 45	238 45	-298 45	-532 45	-4757 45	-1131 45	-4582 45	-601 45	-2241 45	-938 45
	1160 394	-3471 394	-3054 394	-2398 393	-2864 394	-4131 394	-4180 394	-6634 394	-4173 394	-5458 394	-4533 394	-5237 394	-4207 394	-1471 394	1911 394
	877 275	1660 275	47 275	-1209 274	198 275	-3416 275	-779 275	-6303 275	-2720 275	-4990 275	-1006 275	-4850 275	-893 275	798 275	922 275
	-3153 -720	-2934 -720	-2539 -720	-2313 -721	-2359 -720	1415 -720	-3613 -720	-3511 -720	-347 -720	-2896 -720	-442 -720	1877 -720	-3532 -720	-3585 -720	-3651 -720
	-1437 -466	-1696 -466	-3462 -466	-3036 -465	-3281 -466	530 -466	767 -466	-2106 -466	-252 -466	-1692 -466	-893 -466	280 -466	-1771 -466	-2336 -466	-4575 -466
	-444 210	-556 210	-1203 210	1835 209	-1020 210	-731 210	636 210	-6490 210	2534 210	-837 210	356 210	-4980 210	738 210	-2283 210	-621 210
	-1448 -626	-3887 -626	-3512 -626	-3062 -621	-3332 -626	-2156 -626	-4547 -626	401 -626	-4595 -626	369 -626	-179 -626	2181 -626	-140 -626	-650 -626	-1718 -626
	2881 106 -192	-2042 106 -50	654 106 -37	-1286 105 -539	-1433 106 -711	385 106	-2749 106 -1378	-5389 106 -1378	-96 106 -1378	-3923 106 -1378	-3193 106 -1378	-4093 106 -1378	2442 106 -1378	1139 106 -1378	-2623 106 -1378
	1116 399 -3008	2833 399 -4876	-909 399 -5316	2933 402 -1681	2888 399 -1361	-4071 399 -33	-4088 399 -701	-6725 399 -701	1479 399 -701	-5424 399 -701	-4457 399 -701	-5204 399 -701	-4116 399 -701	2082 399 -256	473 399 -701
	-4364 -381 -1115	-4146 -381 -1115	-3765 -381 -1115	-3294 -381 -603	205 -381 -1115	-2609 -381 -1115	944 -381 -894	-4006 -381 -1115	-4853 -381 -1115	2582 -381 -1115	-142 -381 -1115	1863 -381 -1115	-448 -381 -1115	-4797 -381 -1115	-1166 -381 -1115
	1067 43 -894	-1714 43 -894	-230 43 -894	-1096 42 -1549	57 43 -894	-3565 43 -894	147 43 -894	-6801 43 -894	83 43 -894	-5598 43 -894	1600 43 -894	-5354 43 -894	-178 43 -894	-332 43 -894	-302 43 -894
	903 233 -2216	497 233 -1801	-1830 233 -257	-1160 232 -2301	-66 233 -10494	-868 233 -11301	-1312 233 -12065	-7273 233 -12065	-2961 233 -12065	-6172 233 -12065	-3807 233 -12065	-5979 233 -12065	1256 233 -4840	391 233 -12014	1178 233 -6015
	139 -500 -10488	335 -500 -10207	-3448 -500 -9720	-2189 -500 -761	-3267 -500 -9452	582 -500 -10259	-272 -500 -11023	-522 -500 -11023	-4545 -500 -11023	-3714 -500 -11023	-44 -500 -11023	192 -500 -11023	856 -500 -11023	-4492 -500 -10972	-653 -500 -11023
79	-660 -149 -351	-1185 -149 -490	961 -149 -2627	-1425 -150 -2273	77 -149 -3	627 -149 -2	-998 -149 -1	-4871 -149 -1	7 -149 -1	-1905 -149 -1	-491 -149 -1	-381 -149 -1	-1360 -149 -52	-1767 -149 -1	-166 -149 -23

Table 3, Sheet 7/9

Table 3, Sheet 8/9

Upper data block (row labels 100–131; each cell = value / flag):

Row													
100	596 / -249	858 / -294	-4174 / -369	-1359 / 117	843 / 359	-1193 / 96	-798 / 45	-510 / 394	1595 / 275	-3641 / -720	-4568 / -466	-482 / 210	-4623 / -626
101	328 / -249	3290 / -294	-3014 / -369	-1862 / 117	-519 / 359	-1644 / 96	777 / 45	1197 / 394	165 / 275	-2483 / -720	-1191 / -466	1102 / 210	-3462 / -626
102	-3490 / -249	-4239 / -294	-3687 / -369	-2688 / 117	1632 / 359	-2648 / 96	-1914 / 45	3097 / 394	170 / 275	-3279 / -720	-4097 / -466	-2097 / 210	-4084 / -628
103	1665 / -249	-3297 / -294	-2730 / -369	1287 / 117	-1531 / 359	-1372 / 96	700 / 45	-2717 / 394	-1260 / 275	-2201 / -720	-3125 / -466	424 / 210	-3174 / -626
104	-2597 / -255	-3460 / -269	-2876 / -364	-1592 / 117	-1329 / 364	-1653 / 90	-619 / 42	-2265 / 388	1657 / 274	-2473 / -726	-3273 / -470	-969 / 210	-3362 / -629
122	-2336 / -249	-3010 / -294	-447 / -369	-1317 / 117	-1265 / 359	180 / 96	1522 / 45	-2450 / 394	420 / 275	-1912 / -720	-799 / -466	1001 / 210	-2870 / -626
123	-5952 / -249	-6723 / -294	-5843 / -369	-238 / 117	-491 / 359	-955 / 96	-467 / 45	-5159 / 394	-3559 / 275	-5824 / -720	-6569 / -466	-4334 / 210	-6619 / -626
124	-2314 / -249	-2901 / -294	40 / -369	-1485 / 117	87 / 359	-1401 / 96	1275 / 45	-2877 / 394	1005 / 275	1100 / -720	-66 / -466	-928 / 210	-2470 / -628
125	1672 / -249	1867 / -294	-2712 / -369	-1671 / 117	-213 / 359	2944 / 96	625 / 45	-2803 / 394	-1366 / 275	-2201 / -720	-902 / -466	293 / 210	-3113 / -626
126	1694 / -249	-3033 / -294	-164 / -369	-1768 / 117	-1850 / 359	-17 / 96	382 / 45	-2999 / 394	348 / 275	-1872 / -720	-2661 / -466	187 / 210	-2494 / -626
127	422 / -249	-3396 / -294	354 / -369	-1882 / 117	-421 / 359	-1759 / 96	-1211 / 45	-3039 / 394	2387 / 275	-2267 / -720	-3119 / -466	-1269 / 210	489 / -626
128	-760 / -249	1747 / -294	651 / -369	-1341 / 117	-904 / 359	-1285 / 96	1076 / 45	1944 / 394	317 / 275	-3460 / -720	-1665 / -466	-1170 / 210	-809 / -626
129	-960 / -249	2895 / -294	-36 / -369	369 / 117	68 / 359	-224 / 96	971 / 45	-4047 / 394	894 / 275	-3514 / -720	-114 / -466	758 / 210	874 / -626
130	579 / -249	-4527 / -294	885 / -369	-317 / 117	-958 / 359	1137 / 96	-2398 / 45	942 / 394	1040 / 275	-430 / -720	-102 / -466	1499 / 210	-949 / -628
131	1188 / -249	2426 / -294	979 / -369	-1548 / 117	-2088 / 359	-1295 / 96	-4178 / 45	-4921 / 394	-4458 / 275	-2395 / -720	1289 / -466	-4523 / 210	-72 / -626

Lower data block (row labels 94–108; values by column):

Row							
94	1661 / 106 / -699	753 / 399 / -1381	585 / -381 / -1115	-686 / 43 / -894	1435 / 233 / -718	-4552 / -500 / -11001	-17 / -149 / -1352
95	1789 / 106	-2897 / 399	-3713 / -381 / -1115	-208 / 43 / -894	-409 / 233	-3393 / -500	990 / -149
96	-36 / 986 / 108 / -34	-5359 / -3320 / 399	1263 / -381 / -1115	21 / 43 / -894	-1734 / 233	-9651 / -4027 / -500	-154 / -2694 / -149
97	751 / 106 / -31	-5443 / -2623 / 399	424 / -381 / -1115	1845 / 43 / -894	230 / 233	-9501 / -3111 / -500	-212 / 590 / -149
98	-998 / 100 / -303	-5540 / -1860 / 401	-3526 / -381 / -1115	1841 / 38 / -4002 / -894	2505 / 238 / -517	-9292 / -3278 / -505	-1738 / 763 / -143
99	-1016 / 106 / -1266	-2398 / -2357 / 399	-3128 / -93 / -381 / -1115	1857 / 43 / -894	-1238 / 238 / -2137	-505 / -1217 / -500	-143 / -1546 / -149
100	-4264 / 106 / -67	-776 / 3521 / 399	-6758 / -381 / -1115	-3281 / 43 / -894	-645 / 233 / -9958	-8916 / -500	-4 / -4244 / -149
101	3746 / 106 / -89	-4472 / -2587 / 399	-2810 / -381 / -1115	-1022 / 43 / -894	-3 / -710 / 233	-10466 / -2628 / -500	-1366 / -1545 / -149
102	-1350 / 106 / -112	-4056 / -2715 / 399	-3394 / -381 / -1115	-1072 / 43 / -894	318 / -10146 / 233	-9104 / -3101 / -500	-4 / -1738 / -149
103	-1599 / 108 / -100	-3745 / -2914 / 399	3110 / -381 / -1115	181 / 43 / -894	-1979 / 318 / -10302	-9260 / 1786 / -500	-4 / -1830 / -149
104	-1627 / 106 / -2999	-3895 / -2937 / 399	-3366 / -381 / -1115	605 / 43 / -894	2145 / -10481 / 233	-9439 / -3147 / -500	-3 / -486 / -149
105	1125 / 106 / -249	-193 / -1006 / 399	-1270 / -381 / -1115	1666 / 43 / -894	-2912 / -10609 / 233	-9567 / -4357 / -500	-3 / -1642 / -149
106	-2614 / 106 / -2047	-2658 / -3955 / 399	-4731 / -381 / -1115	150 / 43 / -894	-5643 / 150 / 233	-10913 / -4422 / -500	-30 / -1173 / -149
107	-372 / 108 / -634	-1620 / 399 / -400	-1688 / -381 / -1115	-578 / 43 / -894	-11927 / -1522 / 233	-10885 / -192 / -500	-1 / -3107 / -149
108	-297 / 108 / -2168	-4869 / 399 / -363	588 / -381 / -1115	485 / 43 / -894	-12038 / 1900 / 233	-10996 / -3194 / -500	-1 / -3341 / -149

	132	133	134	135	136	137	138	139	140	141	142	143	144
	-3839 / -249	-540 / -249	-7065 / -249	-1076 / -249	4259 / -249	-6720 / -249	-3916 / -249	1981 / -249	123 / -249	-101 / -249	-3807 / -249	-3812 / -249	-3803
	-4396 / -294	-4346 / -294	-7702 / -294	-4874 / -294	3313 / -294	-6964 / -294	-4514 / -294	-4725 / -294	-3630 / -294	-4721 / -294	-4488 / -294	793 / -294	-4481
	1173 / -369	530 / -369	-6356 / -369	-4174 / -369	-4009 / -369	-5531 / -369	-1581 / -369	-819 / -369	1867 / -369	1511 / -369	1049 / -389	-3933 / -369	451
	302 / 117	815 / 117	-1693 / 117	-1326 / 117	-4716 / 117	-764 / 117	1002 / 117	-3012 / 117	-536 / 117	847 / 117	-1010 / 117	-1349 / 117	-851
	-865 / 359	-280 / 359	436 / 359	-3761 / 359	-5288 / 359	211 / 359	-3071 / 359	165 / 359	-22 / 359	926 / 359	844 / 359	-74 / 359	781
	1231 / 96	-546 / 96	-6607 / 98	62 / 96	-677 / 96	-6130 / 98	1741 / 96	-156 / 96	-4450 / 96	-2798 / 96	-193 / 96	2020 / 98	-621
	-851 / 45	-2623 / 45	-5224 / 45	-978 / 45	-5379 / 45	733 / 45	-1065 / 45	-1025 / 45	-323 / 45	-474 / 45	482 / 45	-2011 / 45	131
	385 / 394	117 / 394	732 / 394	3834 / 394	-6164 / 394	-5611 / 394	956 / 394	-4141 / 394	-4943 / 394	-308 / 394	-399 / 394	1394 / 394	2430
	-2962 / 275	-1173 / 275	-4538 / 275	-3528 / 275	634 / 275	-1234 / 275	-165 / 275	225 / 275	-4539 / 275	-417 / 275	-2448 / 275	2127 / 275	22
	-3236 / -720	-3176 / -720	-6744 / -720	-3788 / -720	53 / -720	-5828 / -720	-3377 / -720	-3630 / -720	-37 / -720	-3626 / -720	-718 / -720	-3401 / -720	-3384
	837 / -466	1062 / -466	-7537 / -466	-4555 / -466	-2040 / -466	-6710 / -468	-2413 / -466	-2223 / -466	303 / -466	-4549 / -466	-318 / -468	-1184 / -466	-2372
	1662 / 210	-984 / 210	-5967 / 210	-2824 / 210	-5937 / 210	-6057 / 210	2314 / 210	-453 / 210	-4850 / 210	-2291 / 210	321 / 210	-368 / 210	-2058 / 0
	-119 / -626	1060 / -626	-7458 / -626	-1424 / -626	-4081 / -626	200 / -626	-1803 / -626	-4608 / -626	1242 / -626	-1120 / -626	-4370 / -826	-4383 / -626	-4352
	-2903 / 106	-1984 / -2964 / 106	-5445 / 106 / -1378	-3382 / 106 / -1378	-3987 / 106 / -1378	-5868 / 106 / -1378	-141 / 106 / -1378	-2707 / 57 / 106 / -1378	-3764 / 200 / 106 / -1378	368 / 106 / -1378	-259 / 108 / -1378	-2471 / 106 / -1378	-2474
	-4216 / 399 / -420	-4269 / 399 / -701	-1661 / 399 / -701	-4730 / 399 / -701	-1779 / 399 / 540 / -701	6739 / 399 / -701	-4149 / 399 / -701	1140 / 399 / -701	200 / 399 / -701	-355 / 399 / -701	997 / 399 / -701	-1454 / 399 / -701	1423
	-4244 / -381 / -1115	-4152 / -381 / -1115	-7555 / -381 / -1115	-4813 / -381 / -1115	582 / -381 / -1115	-6739 / -381 / -1115	1616 / -381 / -1115	1014 / -381 / -1115	1014 / -381 / -1115	-4851 / -381 / -1115	-4621 / -381 / -1115	-4632 / -381 / -1115	-544
	-1038 / 43 / -894	-678 / 43 / -894	-4419 / 43 / -894	-1187 / 43 / -894	6355 / 43 / -894	-6125 / 43 / -894	-2567 / 43 / -894	592 / 43 / -894	-5055 / 43 / -894	1251 / 43 / -894	-356 / 43 / -894	-525 / 43 / -894	-626
	-3254 / 233 / -12046	-1481 / 233 / -12065	3854 / 233 / -12065	-3848 / 233 / -12065	-6779 / 233 / -12065	-6209 / 233 / -12036	-3118 / 233 / -12036	70 / 233 / -12036	-5691 / 233 / -12036	432 / 233 / -11773	704 / 233 / -11773	1556 / 233 / -11773	113
	-4097 / -500 / -11003	-4030 / -500 / -11023	-5414 / -500 / -11023	-4583 / -500 / -11023	-428 / -500 / -11023	-4607 / -500 / -11023	-4254 / -500 / -10994	-4540 / -500 / -10994	976 / -500 / -10994	-4535 / -500 / -10731	40 / -500 / -10731	-4311 / -500 / -10731	-4293
10 9	-117 / -149 / -1	1344 / -149 / -1	-4562 / -149 / -1	375 / -149 / -1	-4784 / -149 / -1	3042 / -149 / -1	-1554 / -149 / -1	1683 / -149 / -1	435 / -149 / -1	116 / -149 / -1	89 / -149 / -1	-2839 / -149 / -1	-2838

Table 3, Sheet 9/9

Table 4 Information for producing the HMM for the Pfam alpha-amylase domain (PF 00128)

```
HMMER   2.0 [2.3.1]
NAME    alpha-amylase
ACC     PF00128
DESC    Alpha amylase, catalytic domain
LENG
ALPH    Amino
RF      no
CS      no
MAP     yes
COM     hmmbuild -F HMM_ls.ann SEED.ann
COM     hmmcalibrate --seed 0 HMM_ls.an
NSEQ    54
DATE
CKSUM   2797
GA      -82.0 -82.0
TC      -81.7 -81.7
NC      -82.7 -82.7
XT      -8455    -4  -1000  -1000  -8455    -4  -8455    -4  -8455    -4
NULT      -4  -8455
NULE     595  -1558    85   338  -204   453  -1168   197   249   802  -1085  -142   -21  -313    45   531   201   384  -1098  -644
```

HMM emission scores (match line, insert line per node):

Node		A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
1	match	-1605	-4092	285	925	969	-202	244	-4163	-1052	-4108	-3181	-2229	-3686	3256	-723	-566	423	-3713	766	-3593
	insert	-149	-500	233	43	-381	399	106	-826	210	-468	-720	275	394	45	96	359	117	-369	-294	-248
2	match	-2887	-2711	-5230	-4597	-2673	-1822	-3316	3045	-4195	522	-1917	-883	-4488	-3920	-3998	-1684	-306	1681	-3182	-282
	insert	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-248
3	match	-1209	-2710	-5229	-4593	2368	-4432	278	1011	-4188	444	1689	-4078	-4481	-3811	-3988	-3516	516	317	-3167	2755
	insert	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
4	match	-275	-2705	-5221	-4585	448	-1450	-3297	-30	-4181	-155	899	-4071	2650	-3804	-3982	-1376	1224	1419	-3163	-2821
	insert	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
5	match	-668	-4093	2249	318	-4414	-1377	2291	-4165	-28	-4109	-3182	-743	-954	1554	1650	-2500	-348	-1759	-4276	-3593
	insert	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
6	match	-472	-3803	-3114	-943	-4039	851	-2739	-3669	-2370	385	-3045	-2815	-4109	-2392	2651	1729	-2801	-3408	-4184	328
	insert	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249

HMM state transition scores:

Node	m->m	m->i	m->d	i->m	i->i	d->m	d->d	b->m	m->e
begin	-8455	*	-5356	-1000	*	-8455	*	-8455	*
1	-28	-10485	-5739	-894	-1115	-701	-1378	-36	*
2	-2	-10458	-11500	-894	-1115	-427	-1963	*	*
3	-1	-10485	-11527	-894	-1115	-701	-1378	*	*
4	-1	-10485	-11527	-894	-1115	-701	-1378	*	*
5	-1	-10485	-11527	-894	-1115	-701	-1378	*	*
6	-1	-10485	-11527	-894	-1115	-701	-1378	*	*

Table 4, Sheet 1/32

	7	8	9	10	11	12	13	14	15	16	17	19	21	22	23
	-3409 / -249	1332 / -249	-3210 / -249	686 / -249	-169 / -249	-3242 / -249	-3279 / -249	-3268 / -249	-4077 / -249	822 / -249	-2237 / -250	-1714 / -247	2020 / -249	2948 / -249	-5570 / -249
	-4250 / -294	-4275 / -294	4434 / -294	-3874 / -294	-3868 / -294	1820 / -294	-3961 / -294	-3940 / -294	-4846 / -294	-3081 / -294	-2919 / -295	-2362 / -295	-1558 / -294	3621 / -294	-5743 / -294
	-3568 / -369	-1924 / -369	-3332 / -369	-3313 / -369	-922 / -369	-893 / -369	-3398 / -369	-963 / -369	-864 / -369	-2523 / -389	-2356 / -370	187 / -367	-1532 / -369	-1631 / -389	-5981 / -369
	-4659 / 117	-68 / 117	-500 / 117	-208 / 117	-195 / 117	1384 / 117	587 / 117	-130 / 117	-3056 / 117	329 / 117	635 / 116	1192 / 117	-1094 / 117	-1276 / 117	-4785 / 117
	-4986 / 359	119 / 359	-673 / 359	-222 / 359	-35 / 359	1520 / 359	1690 / 359	-2195 / 359	458 / 359	-1313 / 359	1298 / 358	1266 / 359	1211 / 359	-1378 / 359	-4656 / 359
	-6161 / 96	382 / 96	1149 / 96	170 / 96	-2232 / 98	-889 / 96	669 / 98	-2039 / 96	-2969 / 96	43 / 98	656 / 95	-576 / 97	-1099 / 98	1164 / 96	-411 / 96
	-5797 / 45	544 / 45	-1408 / 45	228 / 45	-1695 / 45	-1519 / 45	-1479 / 45	-463 / 45	102 / 45	970 / 45	1050 / 44	-31 / 45	-661 / 45	-787 / 45	-4302 / 45
	-6017 / 394	-3686 / 394	-3300 / 394	-3285 / 394	-3528 / 394	2148 / 394	-671 / 394	-3381 / 394	-3945 / 394	2468 / 394	318 / 393	-1889 / 393	2208 / 394	-2490 / 394	-5444 / 394
	-5813 / 275	1248 / 275	1805 / 275	504 / 275	654 / 275	-232 / 275	1965 / 275	2669 / 275	1888 / 275	1351 / 275	-875 / 278	1334 / 275	-983 / 275	-1188 / 275	-4630 / 275
	-3124 / -720	425 / -720	-2800 / -720	-2780 / -720	-489 / -720	-2794 / -720	-2866 / -720	-2841 / -720	-3801 / -720	-1990 / -720	-1825 / -721	-1251 / -721	-1184 / -720	-1264 / -720	-5801 / -720
	-1435 / -466	-4107 / -466	-3725 / -466	-3707 / -466	-919 / -466	-3694 / -466	-916 / -466	-1468 / -466	-4870 / -466	-2915 / -466	-1002 / -467	-2136 / -464	-1890 / -466	-1991 / -466	-6413 / -466
	-6493 / 210	1059 / 210	-229 / 210	799 / 210	-1748 / 210	862 / 210	123 / 210	276 / 210	-2381 / 210	551 / 210	-479 / 212	-79 / 211	-717 / 210	-820 / 210	-3824 / 210
	-946 / -626	-4163 / -626	-3781 / -626	-3763 / -626	-3520 / -626	-873 / -626	-948 / -626	-181 / -626	-4742 / -626	-2970 / -626	-2804 / -627	-2122 / -623	-1776 / -626	-1843 / -626	-6660 / -626

	C1	C2	C3	C4	C5	C6	C7
7	-4848 / 106	-1492 / 399	4401 / -381	-6833 / 43	-7039 / 233	-4497 / -500	-1460 / -149
8	301 / 106	-3593 / 399	-4412 / -381	1414 / 43	-2467 / 233	-4092 / -500	1015 / -149
9	-1867 / 106	-3204 / 399	-4030 / -381	381 / 43	1318 / 233	-3710 / -500	-798 / -149
10	-1850 / 106	2378 / 399	-4012 / -381	139 / 43	95 / 233	-3691 / -500	-754 / -149
11	-2112 / 106	-1144 / 399	-3837 / -381	-531 / 43	3319 / 233	-3619 / -500	-2420 / -149
12	-1966 / 106	-3303 / 399	-3978 / -381	-1650 / 43	-489 / 233	-3694 / -500	-1036 / -149
13	-1939 / 106	-3280 / 399	-4097 / -381	-400 / 43	1225 / 233	-3777 / -500	-1310 / -149
14	544 / 106	1478 / 399	-4055 / -381	248 / 43	102 / 233	-3694 / -500	-2309 / -149
15	-2603 / 106	-3666 / 399	-4951 / -381	-356 / 43	3087 / 233	-4662 / -500	507 / -149
16	849 / 106	-2404 / 399	-3222 / -381	454 / 43	-1279 / 233	-2900 / -500	-1432 / -149
17	-898 / 105	-1 / 400	-3055 / -381	858 / 45	-2818 / 145	-8976 / -500	-224 / -149
18	2069 / 105	-1787 / 398	-2409 / -381	725 / 42	-672 / 232	-2136 / -500	-806 / -149
19	2497 / 106	-2079 / 399	-1107 / -381	-809 / 43	-1203 / 233	-1851 / -500	-1101 / -149
20	-977 / 106	-2393 / 399	-1511 / -381	-881 / 43	880 / 233	-2091 / -500	456 / -149
21	931 / 106	3702 / 399	-6164 / -381	-4758 / 43	-4752 / 233	-5007 / -500	-4548 / -149

Table 4, Sheet 2/32

Table 4, Sheet 3/32

	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
24	740 / -149 / -158	-2742 / -500 / -8772	752 / 233 / -3303	-578 / 43 / -894	1125 / -381 / -1115	-55 / 399 / -5017	1540 / 106 / -45	-2808 / -626	674 / 210	-2756 / -466	-1832 / -720	332 / 275	-2345 / 394	-452 / 45	691 / 96
25	1093 / -148 / -3784	-1981 / -501	783 / 232	-929 / 42 / -19	714 / -374 / -8287	-2349 / 397	1735 / 104 / -43	-79 / -628	-817 / 211	-649 / -465	-1134 / -722	774 / 276	455 / 395	-742 / 44	-1242 / 95
27	1491 / -149 / -5	-2213 / -500 / -8620	1203 / 233 / -9662	-932 / 43 / -894	-2512 / 145 / 399	-5076	-1141 / 106 / -43	-177 / -626	-842 / 210	-2307 / -466	-1499 / -720	-1168 / 275	508 / 394	-775 / 45	-1296 / 98
28	-1203 / -149 / -5	-2674 / -500 / -8620	1203 / 235 / -9662	871 / 43	2992 / -381 / -1115	1087 / 399 / -5076	-833 / 106 / -43	-2742 / -626	-420 / 210	-2689 / -466	-1765 / -720	853 / 275	-2264 / 394	1628 / 45	-929 / 98
29	-1213 / -149 / -5	2805 / -500 / -8620	793 / 233 / -9662	588 / 43 / -894	121 / -381 / -1115	632 / 399 / -5076	-986 / 108 / -43	-1897 / -626	-694 / 210	-2062 / -466	1632 / -720	794 / 275	-2381 / 394	-830 / 45	-1146 / 96
30	339 / -150 / -3784	2653 / -501	576 / 235	-852 / 44	-2518 / -382	-2231 / 397	1886 / 104 / -43	301 / -624	-556 / 211	-751 / -467	-1446 / -722	1155 / 276	-2320 / 394	-504 / 44	1181 / 95
32	-39 / -149 / -1468	-2624 / -500 / -8620	1833 / 233 / -9662	352 / 43 / -894	-2924 / -381 / -1115	-2171 / 399 / -5076	-840 / 106 / -43	315 / -626	-433 / 210	-360 / -466	-1720 / -720	2015 / 275	-2269 / 394	1105 / 45	-939 / 98
33	-1761 / -150 / -2331	-653 / -1657 / 239	-1933 / -890	-1066 / 44 / -19	-1714 / -382 / -6287	1555 / 397	-762 / 104 / -43	-1800 / -627	-73 / 209	-1896 / -465	-1201 / -722	-1120 / 276	-2243 / 395	-520 / 44	2066 / 95
38	-1006 / -149 / -2612	-2546 / -500 / -8490	1900 / 234 / -3073	1847 / 44	-2841 / -433	-1663 / 398 / -3740	-540 / 105 / -81	-2615 / -627	-256 / 211	-2550 / -466	-1674 / -721	-283 / 278	1442 / 393	-116 / 45	1044 / 95
40	-1276 / -149 / -10	-1857 / -500 / -8783	-3073 / 233	-3276 / 43 / -45	-5016 / -3951	-2079 / 399 / -4191	-3114 / 106 / -37	-3699 / -626	-3291 / 210	-3965 / -466	-3211 / -720	-2515 / 275	3791 / 394	-3088 / 45	-3258 / 98
41	1795 / -149 / -567	-2196 / -500 / -7741	-565 / 233	897 / 43 / -894	2705 / -381 / -1115	-1787 / 399 / -5295	-749 / 106 / -76	-2424 / -626	-444 / 210	-2454 / -466	-1592 / -720	1798 / 275	-2035 / 394	-339 / 45	-950 / 98
42	1019 / -146 / -2649	-2549 / -500 / -7741	1220 / 232 / -1643	15 / 43 / -894	-2868 / -381	-1686 / 398 / -4278	-602 / 105 / -38	-2639 / -627	-347 / 213	-2589 / -466	-1721 / -721	1280 / 275	-1948 / 393	2872 / 45	-909 / 95
44	-842 / -151 / -2649	-2187 / -502 / -8527	1083 / 235 / -8164	-73 / 44 / -1375	-5058 / 2670	1600 / 403 / -5334	-538 / 103 / -75	-2418 / -629	-193 / 211	-2392 / -468	-1509 / -723	-373 / 273	-1867 / 398	-113 / 43	1666 / 94
53	-468 / -149 / -15	-1232 / -852 / -1748	-1269 / 233	41 / -2921	-204 / -1367	-4299 / -1561 / 399	-1139 / 108 / -113	2029 / -626	-1215 / 210	-1183 / -468	-541 / -720	-1214 / 275	1812 / 394	-1077 / 45	-1420 / 98
54	844 / -149 / -10	-2120 / -500 / -7122	-541 / 233 / -8164	1228 / 43 / -894	-2414 / -381 / -1115	-1688 / 399 / -3735	-358 / 108 / -416	-2139 / -626	1304 / 210	-2120 / -466	-1225 / -720	1410 / 275	-1784 / 394	86 / 45	-407 / 98

	31	32	33	34	35	36
24	1244 / 359	-1216 / 117	-2362 / -369	-2927 / -294	-2247 / -249	
25	-1294 / 360	1299 / 119	-1479 / -371	-2316 / -296	-1803 / -248	
27	1473 / 359	-1252 / 117	-226 / -369	-2655 / -294	-2101 / -249	
28	525 / 359	1091 / 117	-2295 / -369	2230 / -294	-2175 / -249	
29	-1229 / 359	64 / 117	-1647 / -369	-2434 / -294	1661 / -249	
30	294 / 360	-1135 / 121	-1876 / -369	-2586 / -296	-1989 / -251	
32	-43 / 359	-1140 / 117	-2235 / -369	-2820 / -294	-2149 / -249	
33	-1254 / 358	-1145 / 118	-1562 / -370	4088 / -282	-1308 / -247	
38	-835 / 359	-972 / 117	-2160 / -387	-2724 / -295	-1985 / -250	
40	-1512 / 359	1591 / 117	-2799 / -369	-4068 / -294	-3935 / -249	
41	-891 / 359	1554 / 117	-1990 / -369	-2686 / -294	-2033 / -249	
42	-878 / 359	-1019 / 117	-2187 / -370	-2775 / -295	-2035 / -244	
44	1096 / 357	-836 / 121	-1956 / -372	-2579 / -297	-1901 / -236	
53	1463 / 359	-613 / 117	-493 / -369	-1794 / -294	-1390 / -249	
54	-628 / 359	-674 / 117	-1730 / -389	3129 / -294	-1660 / -249	

Column headers: 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69

(value/param)	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
	-1818/-249	-2445/-249	-2561/-249	3735/-249	950/-249	-2272/-249	-5752/-249	-3575/-249	1549/-249	1599/-249	-4633/-249	-2848/-249	-2888/-249	-3593/-249	-3602/-249
	2063/-294	-3126/-294	-3236/-294	2202/-294	1995/-294	2156/-294	2842/-294	-4266/-294	4461/-294	1561/-294	-5412/-294	-3190/-294	-3248/-294	-4276/-294	-4284/-294
	-1302/-389	-2566/-369	-2660/-369	-4497/-369	-1457/-369	489/-369	-6847/-369	-3703/-369	-5998/-369	-915/-369	-4841/-369	1462/-369	-2225/-369	-3714/-369	-3724/-369
	-1448/117	-163/117	912/117	-5247/117	56/117	-823/117	-5649/117	-85/117	-6866/117	-2558/117	-1041/117	-2841/117	1925/117	-916/117	-2567/117
	-451/359	54/359	196/359	-5144/359	-2791/359	-2966/359	-5444/359	-92/359	-6607/359	-349/359	-788/359	-1907/359	-1332/359	659/359	-607/359
	-1840/96	1810/96	-1331/96	-5283/96	-3228/96	-3439/96	-6847/96	-2336/96	-6752/96	653/96	-3537/96	-334/96	-1052/96	-122/96	394/96
	-1404/45	543/45	-785/45	-4740/45	415/45	-3280/45	-6630/45	-108/45	-6118/45	2327/45	-2731/45	-3831/45	-3405/45	1818/45	291/45
	-313/394	-2539/394	-2673/394	-5814/394	-3744/394	-3932/394	-6308/394	-3841/394	-7210/394	-1010/394	-4479/394	-4500/394	-4369/394	-3686/394	-3694/394
	-22/275	793/275	38/275	-4657/275	-3250/275	-3526/275	-503/275	745/275	-6000/275	1061/275	905/275	-852/275	-1442/275	1507/275	545/275
	-1028/-720	-2033/-720	975/-720	-3879/-720	-1230/-720	-1388/-720	-7095/-720	-3178/-720	-817/-720	-3181/-720	-4372/-720	-384/-720	730/-720	-694/-720	-3191/-720
	1445/-466	-635/-466	-87/-466	-3916/-466	-289/-466	-2012/-466	-7616/-466	-1684/-466	2298/-466	-4107/-466	-5236/-466	705/-466	539/-466	-494/-466	-4117/-466
	-1529/210	1777/210	1910/210	-18/210	-3416/210	-3635/210	-6977/210	-488/210	-7378/210	1546/210	-434/210	-4207/210	-3884/210	197/210	3069/210
	902/-626	-3015/-626	-3095/-626	-4407/-626	-1531/-626	1190/-626	-7798/-626	-4154/-626	-5780/-626	-1675/-626	-5311/-626	3058/-626	2217/-626	-4164/-626	-4174/-626

Left block (rows 37–51), each cell shown as value / param1 / param2:

row							
37	590/-149/-4	-1848/-500/-8953	1080/233/-9995	-1680/43/-894	-1869/-381/-1115	-153/399/-3914	-1528/108/-99
38	-1473/-149/-4	-2944/-500/-8047	660/233/-10089	-772/43/-894	-3266/-381/-1115	-2446/399/-3200	1929/106/-166
39	-1602/-149/-4	-3045/-500/-8047	1094/233/-10089	301/43/-894	197/-381/-1115	-2580/399/-3200	1251/106/-54
40	-360/-149/-4	-4540/-500/-9230	-10272/233	-6279/43/-894	3176/-381/-1115	-5925/399/-4772	-2337/106/-54
41	-2207/-149/-4	3448/-500/-9230	-4383/233/-10272	-3804/43/-894	2865/-381/-1115	-3703/399/-1292	2211/106/-757
42	-2335/-149/-2	1113/-500/-9230	-4673/233/-10272	-4038/43/-894	-357/-381/-1115	-1292/399/-802	2872/413/106
43	-5229/-149/-2	-5553/-500/-10248	-6123/233/-10903	-6490/43/-894	-6106/-381/-1115	3663/399/-2023	-6156/108/-408
44	-2600/-149/-2	-4083/-500/-10288	3357/233/-11290	-1838/43/-894	-4399/-381/-1115	-187/399/-130	346/108/-3539
45	-7012/-149/-1	-5986/-500/-11330	-7498/233/-11527	-7818/43/-894	1401/-381/-1115	-7363/399/-701	936/106/-1378
46	-940/-149/-1	-4092/-500/-10485	196/233/-11527	-331/43/-894	-4412/-381/-1115	-3593/399/-701	-2252/106/-1378
47	-3634/-149/-1	-5231/-500/-10485	826/233/-11527	-129/43/-894	-757/-381/-1115	3120/399/-701	-3144/106/-1378
48	-1108/-149/-1	-2725/-500/-10485	-5245/233/-11527	-4810/43/-894	-1161/-381/-1115	-4452/399/-701	-3325/106/-1378
49	1260/-149/-1	-2803/-500/-10485	-1772/233/-11527	-3941/43/-894	-2771/-381/-1115	-1277/399/-701	-3144/106/-1378
50	-597/-149/-1	-4092/-500/-10485	1714/233/-11527	1104/43/-894	-4413/-381/-1115	-3593/399/-701	-2252/108/-1378
51	-886/-149/-1	-4102/-500/-10485	-1336/233/-11527	653/43/-894	-4423/-381/-1115	-1236/399/-701	1004/108/-1378

Table 4, Sheet 4/32

	70	71	74	75	76	77	86	87	88	89	90	91	92	93	94
	-2850	-3593	4195	-5384	-3595	-3595	-2867	-6686	2078	-3828	69	-6228	2050	-5137	-4145
	-249	-252	-249	-249	-249	-250	-249	-249	-249	-249	-249	-249	-249	-249	-249
	866	-4275	-3633	897	-4277	-4278	863	-7108	-3252	-4510	-3379	-6741	5623	-5182	-4485
	-294	-297	-294	-294	-294	-295	-294	-294	-294	-294	-294	-294	-294	-294	-294
	-2167	-714	-1236	-1773	-1108	-3717	-2194	-6340	2155	-3919	348	2423	-6021	1290	-3381
	-369	-372	-369	-369	-389	-370	-369	-369	-369	-369	-369	-369	-369	-369	-369
	-2804	40	-171	-5889	52	-386	-1516	-4961	-472	2930	-151	-170	-6602	-217	1511
	117	119	117	117	117	118	117	117	117	117	117	117	117	117	117
	-1877	-593	-1575	-7629	-1596	378	-3401	-4650	-3598	-250	-520	-6742	-6378	-6294	2681
	359	360	359	359	359	360	359	359	359	359	359	359	359	359	359
	-3835	-851	-2913	-7081	-2342	-1051	-3763	-5877	-4070	-2610	-3437	-7338	-5787	-6450	-4625
	96	95	96	96	98	95	96	96	96	98	96	96	96	96	96
	-49	-97	-872	-6330	929	435	-3501	-140	-3893	-2038	-533	-7112	1984	-6001	-4323
	45	46	45	45	45	44	45	45	45	45	45	45	45	45	45
	-4423	699	-4014	-7194	-3689	1224	-4397	-5670	-4557	-3879	-4268	-7060	-7002	-6641	1518
	394	393	394	394	394	393	394	394	394	394	394	394	394	394	394
	-3894	840	-1139	-7944	74	1061	-1246	-1046	-4156	1252	-3423	-7004	-5711	-6732	233
	275	275	275	275	275	279	275	275	275	275	275	275	275	275	275
	273	-3181	-2430	-2238	-3183	-3184	2191	-6605	-132	-466	176	-3597	-5291	1608	783
	-720	-713	-720	-720	-720	-721	-720	-720	-720	-720	-720	-720	-720	-720	-720
	2295	-4108	-1492	2880	-4109	-4111	2098	-7311	-2608	-4333	-2818	141	-1756	2037	-1531
	-466	-468	-466	-466	-468	-467	-466	-466	-466	-468	-466	-466	-466	-466	-466
	-1076	-912	-2540	-7642	2745	145	225	-5436	-4267	-694	-3255	-7153	-6009	-6715	-4592
	210	210	210	210	210	211	210	210	210	210	210	210	210	210	210
	695	-1864	-984	2398	-4164	-4167	891	-7338	2081	-4376	-2494	3081	-5892	2298	-3617
	-626	-629	-626	-626	-626	-627	-626	-626	-626	-626	-626	-626	-626	-626	-626
	145	-2252	-591	-6742	830	-2254	560	-5181	-3389	-2487	1204	-7445	-3585	-6044	-4173
	106	103	106	106	106	110	106	106	106	106	106	106	106	106	106
	-1378	-1378	-1378	-1378	-1378	-1378	-1378	-1378	-1378	-1378	-1378	-1378	-1378	-1378	-1378

52	78	2795	-428	-4242	-2710	-4369	145	695	-1076	273
	-149	-500	233	43	-381	399				
	-1	-10485	-11527	-894	-1115	-701				
53	117	-4092	2782	545	-4413	-1720	-2252	-1864	-912	-3181
	-150	-502	240	-383	-383	401				
	-583	-1590	-11527	-327	-2304	-701				
54	-2739	-3261	702	-1552	-1049	-1425	-591	-984	-2540	-2430
	-149	-500	233	43	-381	399				
	-1	-10485	-11527	-894	-1115	-701				
55	-6061	-5428	-8423	-7838	174	-8240	-6742	2398	-7642	-2238
	-149	-500	233	43	-381	399				
	-1	-10485	-11527	-894	-1115	-701				
56	917	-4094	-2468	-714	-4414	570	830	-4164	2745	-3183
	-149	-500	233	43	-381	399				
	-1	-10485	-11527	-894	-1115	-701				
57	-680	-4095	1980	794	-4416	741	-2254	-4167	145	-3184
	-150	-501	234	42	-381	399				
	-31	-5599	-11527	-2807	-223	-701				
58	942	-2775	-1042	-4088	-2738	-4341	560	891	225	2191
	-149	-500	233	43	-381	399				
	-1	-10485	-11527	-894	-1115	-701				
59	-4606	-5401	-3869	-4255	-7234	3748	-5181	-7338	-5436	-6605
	-149	-500	233	43	-381	399				
	-1	-10485	-11527	-894	-1115	-701				
60	-1029	-2769	-5299	-1394	1996	-4510	-3389	2081	-4267	-132
	-149	-500	233	43	-381	399				
	-1	-10485	-11527	-894	-1115	-701				
61	-239	-4233	1152	-2113	-4626	555	-2487	-4376	-694	-466
	-149	-500	233	43	-381	399				
	-1	-10485	-11527	-894	-1115	-701				
62	2623	-2941	-4036	-3461	-2940	1140	1204	-2494	-3255	176
	-149	-500	233	43	-381	399				
	-1	-10485	-11527	-894	-1115	-701				
63	-4777	-4256	-7476	-7167	-4920	-7349	-7445	3081	-7153	-3597
	-149	-500	233	43	-381	399				
	-1	-10485	-11527	-894	-1115	-701				
64	-6746	-5975	-6657	-528	-2190	-7063	-3585	-5892	-6009	-5291
	-149	-500	233	43	-381	399				
	-1	-10485	-11527	-894	-1115	-701				
65	-5029	-4582	-7496	-6959	122	-7061	-6044	2298	-6715	1608
	-149	-500	233	43	-381	399				
	-1	-10485	-11527	-894	-1115	-701				
66	-1289	1372	-5290	-4848	-4047	-4330	-4173	-3617	-4592	783
	-149	-500	233	43	-381	399				
	-1	-10485	-11527	-894	-1115	-701				

Table 4, Sheet 5/32

This page contains a large rotated numerical data table (column headers 95, 96, 97, 98, 99, 100, 101, 104, 105, 106, 107, 108, 109, 110, 114; row labels 67–81).

Table 4, Sheet 6/32

Table 4, Sheet 7/32

Table 4, Sheet 8/32

Column index numbers (printed across top): 154, 155, 156, 157, 158, 159, 160, 161, 164, 165, 166, 167, 168, 169, 170

Row	-149 / -1	-500 / -10485	233 / -11527	43 / -894	-381 / -1115	399 / -701	106 / -1378	-626	210	-466	-720	275	394	45	98	359	117	-369	-294	-249
112	564	-4093	406	1072	-4414	-3593	231	-4165	466	-4109	-3182	1180	-3687	1276	947	1015	790	-3715	-4276	-3593
113	251	-5382	-8381	-7805	-3477	-8030	-6821	-2909	-7602	2757	3310	-7863	-7137	-6322	-7056	-7412	-5820	-1221	-5316	-5560
114	-305	1484	-5265	-4631	-2702	-4472	-3348	2502	-4229	-612	1448	-4118	-4520	-3853	-1047	-966	-987	2286	-3212	-2869
115	884	-4092	1870	-634	-4414	-786	843	-4164	304	-4108	-3182	505	-3686	896	434	1104	225	-1701	-4276	-3593
116	1572	-4092	-316	1390	-4413	-3593	-2252	-4164	1260	-1864	-3181	-565	-3686	-20	1256	-11	240	-709	-4275	-3593
117	2346	3387	-5358	-4725	741	-4568	-3442	-753	-4323	731	1479	-4215	-4607	-3938	-4122	-3656	-120	-1125	-3290	-2956
118	-2653	-4128	-124	-729	-778	-1262	4466	-4200	1226	-4143	-3218	354	-3715	446	-370	-1338	-2593	-3750	-4310	-3626
119	1456	-4094	122	1669	-4415	-398	-2253	-4166	901	-4110	-462	905	-3687	1166	-743	607	-2560	-3716	-4277	-3594
120	905	-3975	-3344	-2721	-4151	-4168	-455	-3757	1571	72	-3119	1224	-4233	-2376	2486	-3147	-3042	-301	-4211	1099
121	-4722	-6205	1052	-3086	-7197	3376	-4350	-7191	-4837	-7077	-6482	1612	-5315	-4063	-5831	-2007	-4937	-6437	-7269	-8213
122	-4922	-4389	-7600	-7267	-4589	-7480	-7387	3162	-7240	-1180	3054	-7139	-7096	-6988	-7325	-6879	-4901	1873	-6437	-6132
123	362	-4119	-1060	-1952	-4444	-3625	-196	-4190	3130	-1561	-3209	-437	-3717	274	1372	-1594	-2591	-3743	-4297	-879
124	-2157	1226	-6830	-6365	-616	6361	-5646	2243	-8139	479	-3205	-6010	-6258	-5902	-6093	-5571	-1543	2891	-5359	-4952
125	-4973	-4556	-7390	-6825	-605	6889	-5739	2833	-6544	187	2572	-6548	-6505	-5815	-6253	-6091	-4885	927	-4941	2516
126	790	-2711	-5231	-4595	719	-745	-3305	-173	-4190	1545	2374	-4079	-4482	-3812	-3990	-3518	-868	1788	-3169	-178

Table 4, Sheet 9/32

Table 4, Sheet 10/32

Column block (headers 171–185)

w₁ / w₂	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185
-249	-8581	-324	-6084	427	-8424	-7887	-30	-6703	1492	-3593	560	-3493	1218	750	-2097
-294	-7444	-3163	-6601	-3192	-7395	-7256	-3328	-8797	140	-4275	-4263	949	4543	-2942	-2785
-369	-9400	2047	3648	1465	-9174	-9493	-2318	-5159	-1825	-1325	-1321	-799	-3487	-2372	-2193
117	-8607	-1213	-1808	-400	-8338	-8931	2538	828	742	-1029	-91	-699	887	-1225	-1103
359	-8688	-1769	-6521	-3541	-8424	-9272	996	2399	621	74	1029	-287	-328	-1150	-1014
96	-8076	-3983	-7177	-4013	-8070	-8145	55	-6089	-643	-903	-2346	-1129	-804	864	-960
45	-7951	-3806	-6870	-3835	-8282	-8576	-3300	-5833	-1802	-7	385	-1753	-1587	-443	-318
394	-7557	-4476	-6944	2203	-7552	-7683	-4375	-5088	954	-3686	-3690	1318	-480	-2307	-2136
275	-7636	-4072	-6853	-4101	4459	-8509	-3660	230	-505	366	437	-848	356	-805	1416
-720	-9042	-213	-3609	909	-8951	-9078	3125	98	-3161	-3181	-3166	-3053	-2959	-1855	2728
-466	-9096	665	-944	-885	-9057	-9076	-2749	-6626	-4081	-4108	-2342	-1650	-3882	-2766	-2593
210	-8307	-4183	-7025	-4212	-8487	-8678	117	-6284	-1843	-625	-691	-923	216	-510	-428
-626	-9750	574	-452	1708	-9858	-9783	-831	-6377	-4123	-4164	-4133	-907	-3930	-2816	-2615

Row block (labels 127–141)

row	(-149)	(-500)	(233)	(43)	(-381)	(399)	(106)	(-626)	(210)	(-466)	(-720)	(275)	(394)	(45)	(96)	(359)	(117)	(-369)	(-294)	(-249)
	(-1)	(-10485)	(-11527)	(-894)	(-1115)	(-701)	(-1378)													
127	-8466	-7310	4232	-7396	-8700	-7098	-7495	-9750	-8307	-9096	-9042	-7636	-7557	-7951	-8076	-8688	-8607	-9400	-7444	-8581
128	1130	-2705	-5224	-4588	2004	-410	-3297	574	-4183	665	-213	-4072	-4476	-3806	-3983	-1769	-1213	2047	-3163	-324
129	-202	-4206	-7392	-7072	-4907	-7157	-7171	-452	-7025	-944	-3609	-6853	-6944	-6870	-7177	-6521	-1808	3648	-6601	-6084
130	-1570	-2728	-5251	-4616	2039	-4455	-3328	1708	-4212	-885	909	-4101	2203	-3835	-4013	-3541	-400	1465	-3192	427
131	-8077	-7138	-7637	-8023	-8516	-7075	-7642	-9858	-8487	-9057	-8951	4459	-7552	-8282	-8070	-8424	-8338	-9174	-7395	-8424
132	-8777	-7286	-8125	-8503	-8089	-7246	5476	-9783	-8678	-9076	-9078	-8509	-7683	-8576	-8145	-9272	-8931	-9493	-7256	-7887
133	-2889	2001	-4429	-3820	-2867	-4315	-3121	-831	117	-2749	3125	-3660	-4375	-3300	55	996	2538	-2318	-3328	-30
134	1475	-4014	-6380	-6508	-6579	1671	-5822	-6377	-6284	-6626	98	230	-5088	-5833	-6089	2399	828	-5159	-8797	-6703
135	293	1078	1810	-245	-4380	493	-2259	-4123	-1843	-4081	-3161	-505	954	-1802	-643	621	742	-1825	140	1492
136	1323	-4092	1113	1711	450	746	-2252	-4164	-625	-4108	-3181	366	-3686	-7	-903	74	-1029	-1325	-4275	-3593
137	-1452	-108	1337	1481	89	609	3017	-4133	-691	-2342	-3166	437	-3690	385	-2346	1029	-91	-1321	-4263	560
138	1341	-3956	208	1481	-1210	685	-2203	-907	-923	-1650	-3053	-848	1318	-1753	-1129	-287	-699	-799	949	-3493
139	459	-3868	-497	-1713	-4183	-2010	-2044	-3930	216	-3882	-2959	356	-480	-1587	-804	-328	887	-3487	4543	1218
140	50	-2754	884	2171	-3064	1269	-892	-2816	-510	-2766	-1855	-805	-2307	-443	864	-1150	-1225	-2372	-2942	750
141	1353	-2588	1260	1130	-2871	-1952	-748	-2615	-428	-2593	2728	1416	-2136	-318	-960	-1014	-1103	-2193	-2785	-2097

	186	195	196	197	198	199	200	201	202	203	204	205	206	207	208
	-1686/-231	-2253/-249	21/-249	-3291/-249	885/-248	1373/-249	-3593/-249	-6/-249	763/-249	1094/-249	-3192/-249	151/-249	1996/-249	-1766/-249	3252/-249
	-2296/-297	-2606/-294	-3893/-294	-3973/-294	-3077/-294	215/-294	-4275/-294	-4099/-294	-4244/-294	-4251/-294	-3875/-294	-3592/-294	-1721/-294	-2296/-294	1226/-294
	1220/-372	-259/-369	225/-369	-688/-369	1238/-369	454/-369	-1243/-369	-486/-369	-3676/-369	-3688/-369	-3314/-369	-452/-369	1038/-369	-6/-369	-3334/-369
	-803/117	-141/117	-72/117	-2257/117	622/117	603/117	-898/117	-2387/117	-297/117	48/117	231/117	247/117	-1269/117	70/117	63/117
	-805/359	-417/359	459/359	-219/359	1858/359	-2853/359	1141/359	1401/359	699/359	-320/359	975/359	336/359	-1846/359	-1181/359	135/359
	-670/97	-3220/96	-91/96	1139/96	-3529/96	959/98	-727/98	-2170/96	-1007/96	1407/96	-174/96	-841/96	505/96	819/98	-2026/98
	1549/46	-2878/45	2002/45	-1492/45	-3244/45	-2359/45	265/45	-1622/45	-614/45	-1771/45	852/45	979/45	-1890/45	-605/45	104/45
	-1971/391	-3816/394	-770/394	1280/394	-4203/394	-3976/394	-297/394	-711/394	-639/394	-3665/394	1163/394	1995/394	-2850/394	-2327/394	-3366/394
	-564/273	-185/275	436/275	-1928/275	-3562/275	1631/275	-242/275	1137/275	924/275	-5/275	2037/275	-1876/275	-2208/275	861/275	1348/275
	-1164/-723	-1354/-720	-2798/-720	-2878/-720	-1830/-720	-2491/-720	628/-720	-3004/-720	-3148/-720	-3156/-720	-2781/-720	-110/-720	-476/-720	-1123/-720	-2816/-720
	-2011/-462	-666/-466	-3723/-466	-3804/-466	-2487/-466	-140/-466	-1722/-466	-788/-466	-1180/-466	-1755/-466	-1337/-466	-369/-466	-1132/-466	-1901/-466	-1250/-466
	1106/210•	-3285/210•	973/210•	784/210•	-3505/210•	-1401/210•	1639/210•	857/210•	1549/210•	447/210•	-225/210•	631/210•	-2150/210•	-674/210•	877/210•
	640/-629•	-133/-628•	93/-628•	-293/-628•	180/-628•	-872/-628•	-4164/-628•	-3982/-628•	-1749/-628•	-4136/-628•	-3764/-628•	-3229/-628•	727/-628•	-179/-628•	-10/-628•

	Col1	Col2	Col3	Col4	Col5	Col6	Col7
142	-555/103/-1200	309/399/-825	-2257/-379/-495	-277/43/-1784	1060/232/-3094	-2043/-502/-370	-863/-149/-3199
143	-2613/106/-577	-3761/399/-1601	3508/-381/-448	-3609/43/-894	-3094/235/-10840	-2153/-500/-9798	491/-149/-2
144	-1875/106/-385	-3216/399/-2093	-4108/-381/-1115	-613/43/-894	906/233/-11084	-3708/-500/-10042	-258/-149/-2
145	-1951/106/-901	-251/399/-1106	-431/-381/-1115	1980/43/-894	737/233/-11175	-3789/-500/-10133	-375/-149/-2
146	279/106/-3484	222/399/-135	814/-381/-1115	-3784/43/-894	527/233/-11340	-2632/-500/-10298	480/-149/-2
147	-299/106/-1378	289/399/-701	-4413/-381/-706	-911/43/-894	-871/233/-11527	-3328/-500/-10485	66/-149/-1
148	-236/108/-1378	652/399/-701	-429/-381/-1161	486/43/-894	-2124/233/-2953	-4092/-500/-10485	735/-149/-201
149	-49/106/-2384	-460/399/-307	-4013/-381/-1115	1114/43/-894	1012/233/-11328	-3914/-500/-10286	-456/-149/-2
150	-2233/106/-776	1035/399/-1265	1786/-381/775	-1902/43/-894	257/233/-11501	-4057/-500/-10459	606/-149/-2
151	-2230/106/-776	1273/399/-1265	-4025/-381/-1115	597/43/-894	1484/233/-1915	-34/-500/-10459	-673/-149/-446
152	-1851/106/-108	-384/399/-3799		-47/43/-894	162/233/-3849	-3692/-500/-10015	199/-149/-105
153	-1859/106/-92	382/399/-4011		-79/43/-894	-2140/233/-762	-3339/-500/-9912	-2161/-149/-1289
154	-1624/106/-44	-2793/399/-5073		1286/43/-894	-3028/233/-3521	-1278/-500/-8629	356/-149/-135
155	-940/106/-1657	-2242/399/-550		-781/43/-894	2293/233/-9542	-1974/-500/-8499	414/-149/-6
156	-1934/106/-2793	-3274/399/-225		40/43/-894	-466/233/-11141	-3722/-500/-10099	-2293/-149/-2

Table 4, Sheet 11/32

208
210
211
212
213
214
216
221
222
223
224
225
226
228
230

Table 4, Sheet 12/32

	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245
	-2785 -249	-3323 -249	2118 -249	1883 -249	-3592 -249	-3592 -249	-4 -249	-203 -249	1322 -249	232 -249	-108 -249	1901 -249	-2177 -249	-197 -249	-2111 -250
	-3467 -294	-4005 -294	-4130 -294	-4209 -294	297 -294	845 -294	481 -284	-4209 -294	4896 -294	-4160 -294	1938 -294	2626 -294	-2606 -294	-914 -294	-2813 -295
	-2903 -369	-604 -369	-1248 -369	-1669 -369	-721 -369	-3713 -369	-2547 -369	-3645 -369	-2623 -369	-805 -369	-3331 -369	-1490 -369	-2378 -369	1288 -369	-2249 -370
	-1754 117	776 117	182 117	685 117	-277 117	-2558 117	1668 117	-261 117	-2582 117	-2465 117	-951 117	-1666 117	-1706 117	-1131 117	-1083 118
	-1696 359	-54 359	-1474 359	602 359	1339 359	-313 359	856 359	-2440 359	-687 359	-30 359	1714 359	-1971 359	-1793 359	-1740 359	1444 360
	84 96	-2070 96	510 96	-221 96	-2340 96	-161 96	-2982 96	-349 96	-2825 96	776 96	-475 96	-2041 96	-125 96	-2179 96	-889 95
	1554 45	-364 45	-215 45	396 45	717 45	-319 45	-985 45	-696 45	-2357 45	-1708 45	-233 45	-1614 45	-700 45	-1948 45	-289 44
	-2882 394	-1235 394	1475 394	-3669 394	1610 394	2452 394	-1550 394	94 394	-3922 394	-3597 394	954 394	-3040 394	-2710 394	-2704 394	818 393
	209 275	2270 275	88 275	-720 275	1312 275	-74 275	1208 275	2707 275	-410 275	1359 275	-183 275	1516 275	-1481 275	-2183 275	1169 274
	-2372 -720	-2911 -720	-3029 -720	-440 -720	-3181 -720	-3180 -720	-2265 -720	-3114 -720	784 -720	-3062 -720	-2800 -720	-1203 -720	-1910 -720	-222 -720	-1732 -711
	-1030 -466	-3838 -466	425 -466	-909 -466	-4107 -466	-2357 -466	-154 -466	-4039 -466	-745 -466	-1018 -466	-3725 -466	-113 -466	-2624 -466	-789 -466	-2643 -467
	-123 210	86 210	-813 210	-267 210	-668 210	293 210	-218 210	-105 210	677 210	665 210	-220 210	374 210	2194 210	-2327 210	768 210
	-342 -626	-3893 -626	-3970 -626	72 -626	-4163 -626	-1208 -626	-2692 -626	-285 -626	-1152 -626	922 -626	-3778 -626	-1618 -626	-2650 -626	451 -626	-2701 -627
	-1448 106 -1333	-1982 106 -1227	-204 106 -1673	662 106 -2104	1865 106 -1378	-2252 106 -1378	-2689 106 -451	52 106 -451	32 106 -330	1233 106 -330	1404 106 -113	3226 106 -45	3935 108 -37	2355 106 -131	1333 105 -71
	2235 399 -729	1173 399 -804	58 399 -543	-3576 399 -382	-1236 399 -701	-955 399 -701	-439 399 -1898	96 399 -1898	-1900 399 -2289	1000 399 -2289	240 399 -3733	-374 389 -5012	-2585 399 -5292	-2639 399 -3526	-1997 402 -4371
	-3601 -381 -1115	-796 -381 -1115	108 -381 -1115	959 -381 -1115	-512 -381 -1115	-4411 -381 -1115	1809 -381 -1115	-4342 -381 -1115	1324 -381 -1115	-4278 -381 -1115	1206 -381 -1115	867 -381 -1115	-2813 -381 -1115	3010 -381 -1115	-2942 -379 -5656
	277 43 -894	-190 43 -894	-964 43 -894	231 43 -894	-28 43 -894	-199 43 -894	-2796 43 -894	-1860 43 -894	-1101 43 -894	488 43 -894	-1543 43 -894	-1929 43 -894	-1434 43 -894	-2703 43 -894	-304 43 -29
	-343 233 -10552	826 233 -11213	212 233 -11399	932 233 -11492	757 233 -11527	1936 233 -4388	-3360 233 -11457	1509 233 -5163	-1026 233 -11416	89 233 -2283	307 233 -780	-2481 233 -971	-2132 233 -8800	-3256 233 -8800	1948 232 -3129
	-3282 -500 -9510	-3822 -500 -10171	1292 -500 -10357	-4012 -500 -10450	-4092 -500 -10485	-451 -500 -10485	1548 -500 -10415	1368 -500 -10415	-3153 -500 -10374	290 -500 -10374	-3710 -500 -10043	-2015 -500 -8785	2872 -500 -7758	2374 -500 -7758	-2630 -501 -343
	545 -149 -3	274 -149 -2	-2513 -149 -2	-416 -149 -2	-1215 -149 -1	87 -149 -72	-145 -149 -2	-122 -149 -42	-2642 -149 -2	-2526 -149 -333	-486 -149 -1263	-1728 -149 -1036	-1792 -149 -10	-1177 -149 -10	-1135 -150 -3365

Table 4, Sheet 13/32

172   173   174   175   176   177   178   179   180   181   182   183   184   185   186

Table 4, Sheet 14/32

col →	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216					
274	-1167 / -149 / -296	-3767 / -500 / -10115	1430 / 233 / -2438	-92 / 43 / -894	-4085 / -381 / -1115	1090 / 399 / -3540	-1937 / 106 / -130	744 / -626	-370 / 210	-1505 / -466	-2857 / -720	-799 / 275	-3371 / 394	2171 / 45	-20 / 96	-727 / 359	-2242 / 117	-3387 / -369	2596 / -294	-82 / -249
275	-923 / -149 / -756	-3533 / -500 / -9821	351 / 233 / -1297	1521 / 43 / -894	-174 / -381 / -1115	-647 / 399 / -2698	604 / 106 / -241	-3605 / -626	-128 / 210	-3549 / -466	-2622 / -720	1763 / 275	-3126 / 394	1841 / 45	-106 / 96	-427 / 359	903 / 117	-3155 / -369	-3716 / -294	-3033 / -249
276	-1724 / -149 / -4	-2850 / -500 / -9215	301 / 233 / -10257	742 / 43 / -894	1449 / -381 / -1115	-339 / 399 / -2964	-1422 / 106 / -198	-2710 / -626	-1096 / 210	-624 / -466	-1979 / -720	399 / 275	-2828 / 394	-1027 / 45	-1580 / 96	-103 / 359	-1666 / 117	-2402 / -369	-3095 / -294	3340 / -249
277	-468 / -149 / -190	1610 / -500 / -9401	-19 / 233 / -3038	-4425 / 43 / -894	2001 / -381 / -1115	-4615 / 399 / -2877	-2413 / 106 / -211	-2723 / -626	-4132 / 210	-2869 / -466	-2368 / -720	895 / 275	-4637 / 394	-3667 / 45	-4029 / 96	-3714 / 359	-3284 / 117	-2664 / -369	1981 / -294	3892 / -249
278	-2272 / -149 / -181	-2368 / -500 / -9392	-3736 / 233 / -3102	-3458 / 43 / -894	-2327 / -381 / -1115	1779 / 399 / -3606	-2737 / 106 / -124	-2113 / -626	-3311 / 210	2182 / -466	-1785 / -720	133 / 275	-3786 / 394	-3050 / 45	-3320 / 96	-2695 / 359	807 / 117	-1983 / -369	-2837 / -294	1349 / -249
279	-379 / -149 / -3	-3074 / -500 / -9296	896 / 233 / -10338	-979 / 43 / -894	-3371 / -381 / -1115	378 / 399 / -653	3695 / 106 / -1458	-3105 / -626	-894 / 210	-3080 / -466	-2170 / -720	989 / 275	-2735 / 394	-852 / 45	307 / 96	-1551 / 359	142 / 117	-2684 / -369	1764 / -294	545 / -249
280	-98 / -149 / -2	2770 / -500 / -10156	198 / 233 / -11198	-691 / 43 / -894	-987 / -381 / -1115	-15 / 399 / -691	429 / 106 / -1394	-3536 / -626	-280 / 210	508 / -466	404 / -720	-2045 / 275	-758 / 394	1464 / 45	-285 / 96	-2285 / 359	18 / 117	-1520 / -369	-3845 / -294	1525 / -249
281	-494 / -149 / -2	-3983 / -500 / -10369	1162 / 233 / -11411	689 / 43 / -894	2729 / -381 / -1115	627 / 399 / -655	-2163 / 106 / -1454	-4043 / -626	-1747 / 210	-3997 / -466	-3075 / -720	1162 / 275	-3596 / 394	-1705 / 45	-60 / 96	57 / 359	-512 / 117	-1245 / -369	-4172 / -294	-3493 / -249
282	-190 / -149 / -2	-222 / -500 / -10445	222 / 233 / -11487	-1870 / 43 / -894	962 / -381 / -1115	-1463 / 399 / -1507	-307 / 106 / -626	-641 / -626	-1194 / 210	1539 / -466	311 / -720	-3876 / 275	-4392 / 394	-1052 / 45	-3814 / 96	-103 / 359	-586 / 117	456 / -369	1616 / -294	1529 / -249
283	643 / -149 / -2	-3274 / -500 / -10445	580 / 233 / -11487	-1132 / 43 / -894	981 / -381 / -1115	-423 / 399 / -1507	-2591 / 106 / -626	-1324 / -626	992 / 210	-154 / -466	-2439 / -720	-659 / 275	-659 / 394	-2344 / 45	-2823 / 96	-138 / 359	-2632 / 117	1464 / -369	387 / -294	833 / -249
284	-19 / -149 / -2	-4057 / -500 / -10445	510 / 233 / -11487	431 / 43 / -894	-699 / -381 / -1115	1974 / 399 / -1507	-2216 / 106 / -626	-4129 / -626	639 / 210	-4073 / -466	757 / -720	-564 / 275	-3651 / 394	16 / 45	176 / 96	1016 / 359	-699 / 117	-3679 / -369	-4240 / -294	-3558 / -249
285	-355 / -149 / -2	-3235 / -500 / -10445	-5099 / 233 / -11487	-1194 / 43 / -894	-2927 / -381 / -1115	-4831 / 399 / -1507	-3651 / 106 / -626	-2497 / -626	-4185 / 210	2474 / -466	2254 / -720	-4298 / 275	-4853 / 394	2186 / 45	-4129 / 96	-3927 / 359	-3287 / 117	-2567 / -369	-3603 / -294	423 / -249
286	1739 / -149 / -1	-3082 / -500 / -10485	-182 / 233 / -11527	-3038 / 43 / -894	-3102 / -381 / -1115	-1439 / 399 / -701	-2184 / 106 / -1378	-922 / -626	-2878 / 210	-1755 / -466	-2270 / -720	-3124 / 275	3169 / 394	-2744 / 45	-1266 / 96	-450 / 359	-2728 / 117	-1518 / -369	-3492 / -294	-302 / -249
287	-4651 / -149 / -1	464 / -500 / -10485	3855 / 233 / -11527	-3094 / 43 / -894	-6927 / -381 / -1115	-4683 / 399 / -701	-4206 / 106 / -1378	-8772 / -626	710 / 210	-6721 / -466	-6082 / -720	256 / 275	-5270 / 394	-3898 / 45	-4984 / 96	-4424 / 359	-4816 / 117	-1142 / -369	-6886 / -294	-5983 / -249
288	-5461 / -149 / -1	-4986 / -500 / -10485	-7898 / 233 / -11527	-7183 / 43 / -894	1022 / -381 / -1115	-7248 / 399 / -701	-5463 / 106 / -1378	1643 / -626	-6862 / 210	2782 / -466	-2327 / -720	-8796 / 275	-6720 / 394	-5888 / 45	-8445 / 96	-6466 / 359	-5335 / 117	-654 / -369	987 / -294	-980 / -249

Table 4, Sheet 15/32

Columns: 289, 290, 291, 292, 293, 294, 304, 305, 306, 307, 308, 309, 310, 311, 315

289	290	291	292	293	294	304	305	306	307	308	309	310	311	315
-3587 / -249	2080 / -249	-264 / -249	-3559 / -249	-3629 / -249	1832 / -246	-3877 / -249	1201 / -249	-3593 / -249	1757 / -249	1633 / -249	2186 / -249	-357 / -249	2148 / -250	-2821 / -249
797 / -294	3158 / -294	-4275 / -294	-4239 / -294	880 / -294	-4275 / -281	-4243 / -294	-3297 / -294	-4276 / -294	-4275 / -294	417 / -294	-3509 / -294	-4275 / -294	4062 / -295	-3163 / -294
-3687 / -369	-2176 / -369	-3714 / -369	-3680 / -369	-3751 / -369	257 / -366	3184 / -369	1420 / -369	-3715 / -369	-587 / -369	517 / -369	149 / -369	-3713 / -369	-2858 / -370	1575 / -369
-2568 / 117	2462 / 117	-536 / 117	-2529 / 117	1292 / 117	188 / 116	-445 / 117	-2751 / 117	-1255 / 117	-519 / 117	-1544 / 117	463 / 117	88 / 117	-325 / 116	-956 / 117
-651 / 359	-1878 / 359	-490 / 359	839 / 359	-300 / 359	-13 / 360	-4538 / 359	-714 / 359	210 / 359	-2500 / 359	-3507 / 359	-679 / 359	-1480 / 359	670 / 360	-319 / 359
-517 / 96	-3946 / 96	545 / 96	-653 / 96	-2379 / 96	-1255 / 93	-5031 / 96	2202 / 96	695 / 96	408 / 96	-3976 / 96	-237 / 96	-309 / 96	-1991 / 95	-3981 / 96
-1810 / 45	885 / 45	723 / 45	-1757 / 45	-1826 / 45	771 / 43	-4849 / 45	2287 / 45	1796 / 45	679 / 45	-626 / 45	-1024 / 45	-1792 / 45	-1457 / 44	-3803 / 45
-3699 / 394	-955 / 394	-3686 / 394	-3651 / 394	2711 / 394	-1618 / 393	-5394 / 394	-4274 / 394	-3686 / 394	-3686 / 394	-4473 / 394	-1626 / 394	-3686 / 394	-3276 / 393	-4475 / 394
3227 / 275	-4017 / 275	619 / 275	3577 / 275	971 / 275	-306 / 280	-5054 / 275	-3487 / 275	1775 / 275	354 / 275	-273 / 275	-1261 / 275	1611 / 275	-1883 / 275	-4070 / 275
-440 / -720	-193 / -720	-3181 / -720	54 / -720	-3220 / -720	-413 / -723	-2606 / -720	460 / -720	-3182 / -720	113 / -720	2195 / -720	245 / -720	-3180 / -720	-2425 / -721	2050 / -720
4082 / -466	-71 / -466	-116 / -466	-4072 / -466	-4146 / -466	-2472 / -466	-839 / -466	-2725 / -466	-4109 / -466	-1668 / -466	939 / -466	680 / -466	-996 / -466	-3265 / -467	1109 / -466
495 / 210	-4087 / 210	-158 / 210	-279 / 210	-639 / 210	92 / 209	-5173 / 210	-1463 / 210	1025 / 210	366 / 210	-1151 / 210	1468 / 210	1126 / 210	-1511 / 212	-4179 / 210
-1255 / -626	-2256 / -626	-1840 / -626	-4127 / -626	-4201 / -626	-4163 / -625	230 / -626	814 / -626	-992 / -626	-4162 / -626	2094 / -626	-228 / -626	-1262 / -626	-3174 / -627	1273 / -626
-2266 / 106 / -1378	2308 / 106 / -1378	-2251 / 106 / -1378	1588 / 106 / -1378	-2284 / 106 / -1378	-2252 / 103 / -1378	-4429 / 106 / -1378	-3013 / 106 / -1378	-2252 / 106 / -1378	1269 / 106 / -1378	-3293 / 106 / -1378	-2787 / 106 / -1378	-2252 / 106 / -1378	-1863 / 111 / -4734	-3297 / 106 / -1378
-1778 / 399 / -701	-4433 / 399 / -701	396 / 399 / -701	-3554 / 399 / -235	300 / 399 / -701	-513 / 397 / -701	-5405 / 399 / -623	-4213 / 399 / -384	-125 / 399 / -701	-1356 / 399 / -701	-4423 / 399 / -701	-4035 / 399 / -701	-1776 / 399 / -701	-864 / 398 / -55	-146 / 399 / -701
-4380 / -381 / -1115	-19 / -381 / -1115	-4413 / -381 / -1115	-4379 / -381 / -1115	-4450 / -381 / -1115	4412 / -383 / -262	1402 / -381 / -1115	-2846 / -381 / -1115	-4414 / -381 / -1115	-4412 / -381 / -1115	971 / -381 / -1115	-496 / -381 / -1115	4411 / -381 / -1115	473 / -381 / -606	-497 / -381 / -1115
-1938 / 43 / -894	-4455 / 43 / -894	2089 / 43 / -894	-968 / 43 / -894	874 / 43 / -894	1607 / 43 / 234	-5505 / 43 / -894	-3574 / 43 / -894	319 / 43 / -894	2053 / 43 / -894	-4573 / 43 / -894	-1085 / 43 / -894	907 / 43 / -894	1169 / 44 / -1545	-1393 / 43 / -894
2159 / 233 / -11527	-5076 / 233 / -11527	844 / 233 / -11527	-492 / 233 / -4122	30 / 233 / -11442	726 / 234 / -3805	-6066 / 233 / -11420	-4164 / 233 / -11492	1469 / 233 / -11527	-1137 / 233 / -11527	-1848 / 233 / -11527	-3504 / 233 / -11527	2196 / 233 / -1557	320 / 234 / -10929	-5219 / 233 / -11527
-4071 / -500 / -10485	-2760 / -500 / -10485	-4092 / -500 / -10485	432 / -500 / -10485	-4130 / -500 / -10400	-4092 / -502 / -3650	1317 / -500 / -10378	-2865 / -500 / -10450	-4093 / -500 / -10485	-4091 / -500 / -10485	-2706 / -500 / -10485	-3111 / -500 / -10485	-4091 / -500 / -10485	-3300 / -501 / -3705	-2705 / -500 / -10485
-1455 / -149 / -1	-2908 / -149 / -1	-858 / -149 / -1	-2590 / -149 / -86	-1507 / -149 / -2	260 / -147 / -238	-44 / -149 / -44	65 / -149 / -2	-651 / -149 / -2	338 / -149 / -1	-1252 / -149 / -1	806 / -149 / -1	-60 / -149 / -601	130 / -147 / -116	880 / -149 / -1

Row labels (bottom axis): 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231

Table 4, Sheet 16/32

316
317
327
328
329
330
331
332
333
336
337
338
339
340
341

Table 4, Sheet 17/32

Table 4, Sheet 18/32

Row	p2	385	386	387	388	389	390	391	392	393	394	395	396	397	398	410
262	-249	-274	595	-3593	-3593	2415	892	-386	-674	-2826	1098	-3470	2108	-180 (-248)	-2487 (-255)	-2250 (-248)
263	-294	2822	3926	-4276	-4275	3346	884	-4276	-4276	-3168	-4276	-4153	-3084	-2908	-3166 (-284)	-2800
264	-369	-807	403	-1876	-258	-1132	340	-1172	-1728	1488	-3714	-3592	628	938	-2597 (-388)	305
265	117	-1281	-1339	548	-672	-2832	1259	246	-2558	-775	-407	-577	1049	794	-98 (114)	735
266	359	-768	-838	-362	753	-1378	-2960	850	-1517	-61	-1030	334	-2704	443	-265 (356)	1458
267	96/98	1061	-3927	1484	-2339	-3994	1152	-1132	397	-3981	167	69	-2817	-2598	870 (93)	-1496
268	45/41	-3162	1084	1144	934	-1135	-2549	1806	-736	294	-319	821	-2399	-2174	-700 (41)	-980
269	394/393	-4293	-4456	114	-1007	-4487	-1033	-45	-3686	-20	361	178	-3779	-3575	-50 (393)	-2734
270	275/277	-1257	-802	-744	205	-889	-1113	-380	745	-4069	2049	1664	-2778	829	-1138 (277)	-1402
271	-720/-713	-439	-1922	-3182	-3181	283	2307	-431	-3182	2698	-3182	-3059	-1859	890	-2069 (-713)	785
272	-466/-467	-1069	1050	-4109	-4108	142	370	-4108	-4109	982	-4108	-3986	-329	-326	122 (-467)	-2432
273	210/221	-1519	-4078	1899	417	-4194	55	313	1186	-4176	1815	-287	232	1235	794 (221)	-1043
274	-626/-628	319	1036	-4165	708	752	306	-4164	-4165	1337	-4164	-4042	494	-115	-3038	-366

Triple-value rows (value / p2 / p3):

p2	p3	385	386	387	388	389	390	391	392	393	394	395	396	397	398	410
106	-1378	-292	-3269	2926	436	-3300	813	1047	1673	-3299	975	-2129	-274	-80	538	-1339
399	-701	-1439	-539	-3594	-1745	-4438	-3985	-955	-1956	-4428	199	-189	-2532	85	-1158	-4841
-381	-1115	2837	-1028	-4414	-4413	3037	-3218	-4413	-4414	-2664	-4414	-4291	-171	-21	1194	-2635
43	-894	-3594	-4453	-1918	1661	-4599	-1492	912	1709	-4578	-1918	1296	670	-2471	1325	-1835
233	-11527	1150	-1621	-707	580	-5233	-1037	1289	1238	-5213	262	462	-2489	-1066	-1356	-10165
-500	-10485	-2879	-2720	-4093	-4092	-2717	-3183	-4092	-4093	-2709	-4092	-3970	-2676	-2793	-2978	-2494
-149	-1	-1153	122	-233	499	-2891	-503	-159	1274	407	85	1241	-284	625	73	-356

Row labels (bottom, left to right): 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276

Table 4, Sheet 19/32

	411	412	413	414	415	418	417	418	419	420	421	422	423	436	437
	2375 -249	896 -249	-3413 -249	-2830 -249	2512 -249	-208 -249	1386 -249	-5886 -249	-6874 -249	1326 -249	1422 -249	-915 -249	-1999 -237	-3362 -249	-3302 -249
	-3528 -294	-3886 -294	2039 -294	-3175 -294	990 -294	1341 -294	-3246 -294	-6934 -294	-7315 -294	2926 -294	4640 -294	-4273 -294	-2374 -297	-4044 -294	-3983 -294
	-2980 -369	-3325 -369	-1765 -369	1285 -369	-1046 -369	1040 -369	2596 -369	-6301 -369	-7450 -369	2210 -369	-795 -369	-1726 -369	-1213 -372	-1514 -369	-1623 -369
	-1846 117	-2169 117	-2380 117	215 117	-2816 117	1924 117	-218 117	-4888 117	-6009 117	-1307 117	-2816 117	-841 117	1564 115	580 117	446 117
	-1780 359	691 359	50 359	-364 359	-3498 359	-1828 359	-3602 359	-4432 359	-5553 359	-3509 359	-532 359	175 359	1858 358	-635 359	518 359
	1325 96	570 96	-2163 96	692 96	-3958 96	-3955 96	-4072 98	-5293 98	-6414 96	-3981 96	-750 96	-2341 96	-1945 93	-1027 96	-2060 96
	1163 45	426 45	-1615 45	-3728 45	-3771 45	-843 45	-3892 45	1419 45	-4908 45	-3804 45	-3772 45	1361 45	-1561 44	1765 45	-1511 45
	597 394	1981 394	1566 394	129 394	-774 394	-4448 394	-4560 394	-5218 394	-8088 394	-862 394	-1510 394	-3687 394	-2261 395	-3459 394	141 394
	-1452 275	-1839 275	430 275	-4006 275	-4042 275	-1446 275	-4162 275	-3269 275	-1213 275	-1502 275	-4043 275	-1104 275	-1621 278	712 275	1208 275
	-2456 -720	-2792 -720	-3001 -720	1507 -720	2103 -720	1757 -720	207 -720	-6095 -720	-7325 -720	1375 -720	-1913 -720	-3178 -720	-1160 -718	-166 -720	-2887 -720
	-3364 -466	-3719 -466	-3927 -466	448 -466	-1108 -466	304 -466	239 -466	-2189 -466	-7778 -466	-274 -466	-298 -466	-758 -466	-1880 -468	-1941 -466	-1811 -466
	-1072 210	1641 210	326 210	-4079 210	-4136 210	-4155 210	-1723 210	-4391 210	-5596 210	-1866 210	-4138 210	205 210	-1739 211	-1606 210	-1553 210
	-3416 -626	-3775 -626	-3981 -626	893 -626	-1048 -626	1605 -626	290 -626	-6860 -626	-8036 -626	1435 -626	1399 -626	-1843 -626	1572 -627	-3928 -626	-3859 -626
	1510 106 -1028	890 106 -1052	-2074 106 -3599	-3269 106 -1378	185 106 -1378	-3270 106 -2008	-3390 106 -1378	-4075 106 -1378	-5139 106 -1378	101 106 -1378	-349 106 -1378	2467 106 -1378	-1642 103 -2302	-2025 106 -169	-1969 106 -138
	1869 399 -972	-1118 399 -950	505 399 -124	-1166 399 -701	-4417 399 -701	-4398 399 -412	-4516 399 -701	3180 399 -701	-5502 399 -701	-4425 399 -1110	399 -701	1357 399 -701	1184 402 -327	1819 399 -3177	1065 399 -3476
	-3665 -381 -1115	-4024 -381 -1115	-1111 -381 -1115	834 -381 -1115	3229 -381 -1115	109 -381 -1115	2352 -381 -1115	-6920 -381 -1115	-7732 -381 -1115	-167 -381 -1115	1589 -381 -1115	-4408 -381 -1115	-2006 -383 -136	-4178 -381 -1115	-4111 -381 -1115
	-1142 43 -894	430 43 -894	1575 43 -894	-1322 43 -894	-907 43 -894	-4560 43 -894	-4674 43 -894	-1335 43 -894	3932 43 -894	-4585 43 -894	-4530 43 -894	-714 43 -894	-1892 42 -3476	-225 43 -894	-152 43 -894
	239 233 -10389	369 233 -11070	1701 233 -11318	-1863 233 -11527	-348 233 -5610	-5196 233 -11498	-5308 233 -11527	158 233 -11527	-3549 233 -11527	-5221 233 -11527	-1661 233 -11527	1418 233 -217	-2291 235 -8690	1096 233 -4136	1830 233 -269
	-3358 -500 -9346	-3703 -500 -10028	-3912 -500 -10276	71 -500 -10485	-2711 -500 -10485	1323 -500 -10455	-2779 -500 -10485	-6618 -500 -10485	-7026 -500 -10485	-2704 -500 -10485	-2711 -500 -10485	-4088 -500 -10485	-1200 -503 -1741	-3859 -500 -10220	-3794 -500 -10136
277 278 279 280 281 282 283 284 285 286 287 288 289 290 291	-1905 -149 -3	-1093 -149 -2	-976 -149 -2	429 -149 -1	-781 -149 -31	-561 -149 -2	-2959 -149 -1	968 -149 -1	-5864 -149 -1	-242 -149 -1	-1781 -149 -1	162 -149 -2847	-732 -148 -518	588 -149 -86	1059 -149 -2561

Table 4, Sheet 20/32

Table 4, Sheet 21/32

	454	455	456	458	459	460	461	462	463	464	465	466	467	468	469
	-3593 -249	1529 -249	-3531 -245	1234 -249	-2347 -249	-75 -249	-3663 -249	672 -249	1241 -249	1960 -249	1040 -249	588 -249	-1068 -249	493 -249	1408 -249
	-4275 -294	2000 -294	-4212 -295	-3006 -294	-2692 -294	-3096 -294	-4350 -294	1010 -294	-4254 -294	-3603 -294	833 -294	-4266 -294	-4172 -294	-3732 -294	-4273 -294
	-3714 -369	1357 -369	-601 -388	-154 -369	1211 -369	-2072 -369	-3789 -369	1077 -369	-976 -369	-2691 -369	-1647 -369	-3697 -369	662 -369	-2869 -369	-1544 -369
	267 117	-356 117	1338 119	69 117	282 117	249 117	63 117	-2845 117	288 117	-9 117	-780 117	438 117	727 117	-2655 117	-291 117
	939 359	460 359	294 360	916 359	-1354 359	-537 359	712 359	-3535 359	536 359	-1197 359	-279 359	-215 359	-9 359	-1167 359	-102 359
	-1015 96	-3979 96	-2280 95	-392 96	851 96	-3551 96	-609 96	-4005 96	553 96	-1349 96	548 96	384 96	-758 96	341 96	1097 96
	664 45	-3801 45	1352 48	-2126 45	-3272 45	-3268 45	456 45	-3826 45	-427 45	-2491 45	863 45	269 45	158 45	232 45	1891 45
	-1408 394	-4474 394	1056 393	-3585 394	-3982 394	-1479 394	-3743 394	-4499 394	1822 394	-4036 394	-3981 394	-136 394	-3725 394	-3947 394	-883 394
	760 275	1098 275	1235 277	-622 275	-641 275	142 275	1531 275	-851 275	-2242 275	441 275	951 275	-2234 275	-396 275	-1146 275	732 275
	-3181 -720	251 -720	157 -721	2904 -720	1518 -720	2046 -720	-3258 -720	-1932 -720	-3155 -720	93 -720	526 -720	-19 -720	-3056 -720	-542 -720	-237 -720
	-2123 -466	1118 -466	-2296 -487	-1373 -466	1102 -466	532 -466	-4183 -466	-55 -466	-182 -466	1342 -466	-870 -466	-1227 -466	-125 -466	1197 -466	-2211 -466
	-1832 210	-4178 210	-611 209	-795 210	-3631 210	-357 210	-1905 210	-4204 210	397 210	-2594 210	-11 210	141 210	885 210	-574 210	400 210
	-1864 -626	-1012 -626	-1403 -627	-322 -626	1221 -626	-40 -626	-4239 -626	-537 -626	-985 -626	-2850 -626	-1376 -626	-1835 -626	1134 -626	1285 -626	-1261 -626
	414 106 -1378	-3295 108 -1378	-2192 105 -1378	638 106 -189	-2798 106 -1523	-3000 106 -3351	-2314 106 -1378	-3303 106 -1378	503 106 -1378	2505 106 -1378	1857 106 -1378	-2256 106 -1378	-246 106 -1378	603 106 -1378	1186 106 -1378
	-662 399 -701	41 399 -701	-1341 398 -267	104 399 -3026	1013 399 -617	-4167 399 -149	-185 399 -701	-4450 399 -701	-1402 399 -701	347 399 -701	-381 399 -701	984 399 -701	-3634 399 -701	207 399 -701	111 399 -701
	-4413 -381 -1115	-906 -381 -1115	-4348 -382 -5804	1059 -381 -1115	-2191 -381 -1115	3055 -381 -1115	-4487 -381 -1115	3844 -381 -1115	-350 -381 -1115	185 -381 -1115	2102 -381 -1115	883 -381 -1115	573 -381 -1115	-181 -381 -1115	-4408 -381 -1115
	1573 43 -894	-4581 43 -894	874 42 -26	-495 43 -894	-4015 43 -894	-3810 43 -894	1181 43 -894	-4808 43 -894	-68 43 -894	-2730 43 -894	-2584 43 -894	-6 43 -894	-986 43 -894	-2498 43 -894	-4 43 -894
	1457 233 -11527	-5217 233 -4400	108 234 -1503	-2963 233 -10936	-4645 233 -10988	-613 233 -11363	2491 233 -11527	-148 233 -11527	394 233 -11527	1186 233 -11527	-3142 233 -11527	1226 233 -11527	165 233 -11527	529 233 -11527	-2468 233 -11527
	-4092 -500 -10485	-2705 -500 -10485	-4028 -501 -2403	-2610 -500 -9894	-2235 -500 -9944	-2651 -500 -10321	-4167 -500 -10485	-2730 -500 -10485	-4063 -500 -10485	-3224 -500 -10485	-3318 -500 -10485	-4079 -500 -10485	-3951 -500 -10485	551 -500 -10485	-4088 -500 -10485
	819 -149 -1	32 -149 -71	142 -150 -1126	-734 -149 -2	-694 -149 -2	-1055 -149 -2	-467 -149 -1	-1259 -149 -1	-2622 -149 -1	-2746 -149 -1	-203 -149 -1	216 -149 -1	388 -149 -1	346 -149 -1	-41 -149 -1
307															
308															
309															
310															
311															
312															
313															
314															
315															
316															
317															
318															
319															
320															
321															

Table 4, Sheet 22/32

	470	471	472	473	474	476	477	478	479	480	481	482	483	484	485
	-3593 -249	-283 -249	-1010 -249	-2781 -249	-4269 -250	-2402 -248	-1940 -249	743 -249	12 -249	1369 -249	-591 -249	-3188 -249	1210 -249	-3093 -249	1482 -249
	-4276 -294	-3195 -294	-4205 -294	-3452 -294	-4706 -295	-3084 -294	-2366 -294	3483 -294	-3891 -294	773 -294	-4005 -294	-3872 -294	2776 -294	-3776 -294	-2222 -294
	-1228 -369	1440 -369	509 -369	322 -369	-3536 -370	-2520 -369	1115 -369	-585 -369	-626 -369	-1063 -369	5 -369	-3311 -369	-1017 -369	-3214 -369	-1755 -369
	-202 117	400 117	-1331 117	-666 117	-2222 117	-1370 117	-1570 117	155 117	-935 117	-51 117	446 117	857 117	-2075 117	969 117	-720 117
	534 359	20 359	-1242 359	518 359	-1961 359	218 359	-1881 359	-458 359	425 359	-296 359	14 359	1184 359	86 359	1133 359	-685 359
	-205 96	-3855 96	213 96	1381 96	-3520 95	692 96	383 96	-1954 96	751 96	-2483 96	-2091 96	-283 96	471 96	658 96	-91 96
	-720 45	-1121 45	478 45	791 45	-2885 45	1034 45	-1491 45	709 45	1918 45	-2003 45	-1544 45	-206 45	2031 45	752 45	2049 45
	546 394	129 394	-3713 394	-2902 394	3218 393	-2498 394	-2961 394	-3066 394	-3324 394	-3613 394	-3434 394	1787 394	-988 394	-3187 394	-1798 394
	1550 275	-363 275	-2274 275	55 275	2243 277	2083 275	675 275	-1885 275	832 275	-883 275	1912 275	781 275	-1944 275	947 275	2085 275
	-3181 -720	-1944 -720	-3095 -720	-2352 -720	-3838 -714	-1989 -720	1506 -720	-1509 -720	-231 -720	921 -720	-2908 -720	-2778 -720	-2247 -720	613 -720	-1233 -720
	-4108 -466	275 -466	-562 -466	-1378 -466	-4654 -466	-2914 -466	-1854 -466	-441 -466	-1033 -466	672 -466	-3829 -466	-56 -466	-3052 -466	-823 -466	-2097 -466
	1050 210	-3946 210	57 210	-168 210	-3135 210	-846 210	1866 210	-142 210	745 210	-803 210	1051 210	469 210	-1580 210	159 210	1349 210
	-4164 -626	-70 -626	-1596 -626	532 -626	-4556 -627	-2967 -626	-1567 -626	-51 -626	146 -626	-389 -626	-814 -626	-3760 -626	623 -626	-3684 -626	-2144 -626
	44 106	-3231 106	605 106	-1470 106	-2893 105	-1064 106	-1625 106	-1712 106	-1891 106	775 106	412 106	-1848 106	-1891 106	-1752 106	-301 106
	-1378	-1378	-1378	-64	-576	-86	-268	-1079	-243	-537	-154	-107	-157	-90	-64
	575 399	-1982 399	58 399	794 399	-4529 398	1017 399	-2890 399	-1027 399	-1338 399	1948 399	1049 399	-257 399	-582 399	-343 399	-1691 399
	-701	-701	-701	-4529	-1602	-4118	-2560	-925	-2687	-1687	-3306	-3810	-3279	-4043	-4530
	-4414 -381	1303 -381	2076 -381	-3561 -381	-372 -381	456 -381	2376 -381	2146 -381	-920 -381	-3010 -381	-624 -381	-4010 -381	985 -381	-3913 -381	-2376 -381
	-1115	-1115	-1115	-1115	-4787	-1115	-1115	-1115	-1115	-1115	-1115	-1115	-1115	-1115	-1115
	641 43	-1789 43	-1044 43	-1144 43	-2154 43	1025 43	-1796 43	287 43	-245 43	-865 43	-10 43	-269 43	368 43	-676 43	-63 43
	-894	-894	-894	-894	-53	-894	-894	-894	-894	-894	-894	-894	-894	-894	-894
	449 233	-4902 233	572 233	916 233	-1835 232	-42 233	-2372 233	-415 233	-92 233	488 233	367 233	592 233	-173 233	1872 233	-552 233
	-11527	-11527	-1042	-763	-996	-10033	-10099	-10413	-5194	-11103	-3115	-3296	-10898	-273	-1408
	-4092 -500	-2743 -500	-3995 -500	-3258 -500	-2600 -500	891 -500	-1974 -500	-2342 -500	-3702 -500	-2946 -500	-3817 -500	-3689 -500	451 -500	-3592 -500	-2121 -500
	-10485	-10485	-10485	-9527	-1305	-8991	-9057	-9370	-10054	-10061	-10186	-10010	-9856	-9895	-7361
	507 -149	1505 -149	-1181 -149	54 -149	-1841 -149	-1431 -149	-355 -149	-1797 -149	176 -149	-2358 -149	-225 -149	-2215 -149	-1053 -149	-2120 -149	-796 -149
	-1	-1	-961	-1288	-3410	-4	-4	-3	-41	-2	-178	-157	-2	-2547	-697

Table 4, Sheet 23/32

| 322 | 323 | 324 | 325 | 326 | 327 | 328 | 329 | 330 | 331 | 332 | 333 | 334 | 335 | 336 |

Columns 486–500 (each cell shows two stacked values):

486	1779/-249	-1722/-294	-944/-369	-399/117	1020/359	-515/96	-5/45	-1603/394	1518/275	-619/-720	-1405/-466	-83/210	-1234/-626
487	-2045/-249	-2498/-294	-631/-369	-1076/117	2830/359	-2230/96	-2008/45	-2390/394	-1892/275	-1167/-720	-1614/-466	-2146/210	1819/-626
488	-1861/-249	-2518/-294	-1917/-369	1925/117	-823/359	-650/96	1344/45	-1988/394	-531/275	-1422/-720	-2315/-466	861/210	-2325/-626
489	-2707/-249	1250/-294	-456/-369	-784/117	-988/359	15/98	-3673/45	-1379/394	-659/275	3415/-720	926/-466	-4045/210	-215/-626
490	1229/-249	-4261/-294	-1811/-369	-2560/117	313/359	1455/96	250/45	-1426/394	775/275	-3164/-720	-759/-466	1359/210	64/-626
491	58/-249	415/-294	-3714/-369	189/117	-1018/359	137/96	1529/45	-3686/394	766/275	-3181/-720	-942/-466	866/210	-4164/-626
492	733/-249	984/-294	-260/-369	-1133/117	-1988/359	-3978/96	-1394/45	-1693/394	-4067/275	336/-720	2065/-466	-1972/210	630/-626
493	1177/-249	-4186/-294	500/-369	-163/117	-102/359	-921/96	-798/45	-3720/394	1176/275	14/-720	-1722/-466	1865/210	-3951/-626
494	-3593/-249	-4275/-294	-2008/-369	905/117	509/359	-2339/96	1065/45	-3686/394	1669/275	-3181/-720	-2362/-466	194/210	-1477/-626
495	338/-249	2641/-294	-61/-369	-293/117	97/359	504/98	-1143/45	-949/394	279/275	2818/-720	-219/-466	-123/210	115/-626
496	585/-249	990/-294	-343/-369	587/117	-1209/359	-1274/96	-3799/45	-1693/394	-4067/275	1591/-720	616/-466	-1135/210	1948/-626
497	-3539/-249	-4222/-294	-3661/-369	842/117	631/359	-1067/96	791/45	108/394	1250/275	871/-720	-1981/-466	-306/210	-1236/-626
498	379/-249	-4222/-294	-3661/-369	-557/117	861/359	-321/96	489/45	-3633/394	55/275	-3128/-720	-371/-466	777/210	-1212/-626
499	546/-249	4091/-294	802/-369	643/117	-1029/359	-182/96	125/45	-3974/394	-1323/275	1421/-720	-688/-466	-1221/210	-786/-626
500	661/-249	-3791/-294	-531/-369	-832/117	-1306/359	-57/96	2001/45	-3911/394	-572/275	533/-720	327/-466	-308/210	245/-626

Rows 337–351 (each cell shows three stacked values):

337	997/-149/-17	-1241/-500/-6997	-578/233/-8039	-133/43/-894	-1517/-381/-1115	-1399/399/-4345	-332/108/-73
338	-813/-149/-13	-1193/-500/-7341	-2517/233/-8383	-2345/43/-894	-1917/-381/-1115	-1837/399/-3330	-1987/106/-151
339	-927/-149/-8	2366/-500/-8030	1639/233/-9072	-228/43/-894	-2602/-381/-1115	-1871/399/-177	-568/106/-3112
340	1374/-149/-2	-2592/-500/-10357	-5077/233/-11399	-4443/43/-894	-124/-381/-1115	-1761/399/-179	-3175/106/-3103
341	669/-149/-1	-4072/-500/-11399	-2127/233/-11527	-1928/43/-894	-4384/-381/-1115	267/399/-701	-2258/106/-1378
342	-60/-149/-1	-4092/-500/-10485	1793/233/-11527	555/43/-894	-4413/-381/-1115	-721/399/-701	591/106/-1378
343	-2877/-149/-1	521/-500/-10485	-228/233/-11527	-4579/43/-894	1897/-381/-1115	-1418/399/-701	-3294/106/-1378
344	1114/-149/-1	-3970/-500/-10485	600/233/-11527	-1986/43/-894	-1332/-381/-1115	-3628/399/-701	-2293/108/-1378
345	152/-149/-1	-4092/-500/-10485	1011/233/-11527	793/43/-894	-4413/-381/-1115	428/399/-701	44/108/-1378
346	-218/-149/-1	-2875/-500/-10485	-4193/233/-11527	-3609/43/-894	-142/-381/-1115	-1211/399/-701	-323/108/-1378
347	71/-149/-63	-2705/-500/-10485	-5213/233/-11527	-4578/43/-894	495/-381/-1115	657/399/-701	-3294/108/-1378
348	388/-149/-2	-4039/-500/-10485	1017/233/-11466	598/43/-894	-4360/-381/-1115	-547/399/-1793	971/106/-491
349	-1398/-149/-2	-4039/-500/-10423	1347/233/-11466	-135/43/-894	-4360/-381/-1115	1020/399/-287	1623/106/-2470
350	-2724/-149/-1	-3331/-500/-10485	-141/233/-11527	-323/43/-894	272/-381/-1115	-574/399/-701	-2612/106/-1378
351	623/-149/-1	-3456/-500/-10485	-2864/233/-11527	-82/43/-894	-57/-381/-1115	615/399/-701	-2532/106/-1378

Table 4, Sheet 24/32

Table 4, Sheet 25/32

	501	502	503	504	505	507	509	510	511	512	513	514	515	516	517
	879 / -249	789 / -249	2373 / -249	-654 / -249	918 / -250	-2585 / -250	-3113 / -249	949 / -249	-879 / -249	-3475 / -249	-2821 / -249	-2881 / -249	1759 / -249	-3993 / -249	-3585 / -249
	258 / -294	1504 / -294	139 / -294	353 / -294	-4189 / -295	-3264 / -280	-3794 / -294	1301 / -294	3343 / -294	-4086 / -294	-3163 / -294	2337 / -294	2012 / -294	2295 / -294	-4260 / -294
	-3709 / -389	-783 / -369	-420 / -369	-1328 / -369	-3628 / -370	1299 / -370	837 / -389	-1379 / -369	259 / -369	-1175 / -389	2404 / -389	1278 / -369	69 / -369	2430 / -369	-1474 / -369
	341 / 117	-337 / 117	-226 / 117	360 / 117	-2472 / 120	1125 / 117	153 / 117	-291 / 117	-349 / 117	-297 / 117	358 / 117	2587 / 117	-8467 / 117	-3937 / 117	-1084 / 117
	1019 / 359	426 / 359	-214 / 359	1170 / 359	633 / 359	-1502 / 359	-915 / 359	-647 / 359	-409 / 359	539 / 359	-885 / 359	-463 / 359	-6411 / 359	-4820 / 359	-1034 / 359
	-1267 / 98	552 / 96	-391 / 98	-573 / 98	-2253 / 95	362 / 96	-184 / 96	-61 / 96	940 / 96	-721 / 98	-1508 / 98	-4031 / 86	-6595 / 96	-5204 / 96	-909 / 96
	-39 / 45	-71 / 45	-3674 / 45	-213 / 45	-668 / 51	698 / 45	704 / 45	932 / 45	-635 / 45	1155 / 45	-3797 / 45	-3850 / 45	-729 / 45	-4940 / 45	-1810 / 45
	-3687 / 394	-3694 / 394	1019 / 394	2162 / 394	-795 / 395	-210 / 393	799 / 394	-3388 / 394	-3689 / 394	-3762 / 394	-4473 / 394	-4523 / 394	-7055 / 394	-5603 / 394	-3698 / 394
	-11 / 275	60 / 275	-3959 / 275	151 / 275	1912 / 276	-1232 / 278	1357 / 275	-729 / 275	1163 / 275	1147 / 275	508 / 275	1891 / 275	-5931 / 275	-5350 / 275	-230 / 275
	690 / -720	1388 / -720	1708 / -720	1182 / -720	-3095 / -721	-2168 / -721	-2699 / -720	-2788 / -720	-3172 / -720	1615 / -720	480 / -720	-1957 / -720	-4871 / -720	186 / -720	-3161 / -720
	-991 / -466	-549 / -466	449 / -466	-4087 / -466	-633 / -467	-3090 / -466	-3624 / -466	-939 / -466	-1007 / -466	256 / -466	-12 / -466	-66 / -466	426 / -466	1541 / -466	-4079 / -466
	319 / 210	188 / 210	-886 / 210	-667 / 210	-92 / 211	-838 / 210	895 / 210	-501 / 210	1670 / 210	-1980 / 210	-4171 / 210	-4221 / 210	-7161 / 210	-5425 / 210	-1852 / 210
	-4157 / -626	-4109 / -626	-627 / -626	-4142 / -626	-4077 / -627	-3138 / -627	-1392 / -626	-1477 / -626	-1474 / -626	-3729 / -626	500 / -626	366 / -626	-5428 / -626	1828 / -626	-1160 / -626

352	486 / 106 / -1378	748 / 399 / -701	1032 / -381 / -1115	301 / 43 / -894	25 / 233 / -11527	-4088 / -500 / -10485	-156 / -149 / -1
353	-2261 / 106 / -1378	438 / 399 / -701	231 / -381 / -1115	-302 / 43 / -894	421 / 233 / -11527	-4061 / -500 / -10485	386 / -149 / -1
354	1302 / 106 / -1378	-465 / 399 / -701	-578 / -381 / -1115	-1583 / 43 / -894	-4984 / 233 / -5900	1409 / -500 / -10485	-879 / -149 / -1
355	525 / 106 / -1378	-438 / 399 / -701	-4392 / -381 / -1115	-90 / 43 / -894	789 / 233 / -11527	-4071 / -500 / -10485	-1603 / -149 / -1
356	930 / -799 / -1768	15 / -1234 / -502	-4327 / -382 / -5935	874 / 42 / -24	-501 / 232 / -739	-4006 / 1385	164 / -148 / -2406 / -285 / -78 / -10461 / -4264
357	-1254 / -860	-16 / -1155	-3392 / -4624	959 / -60	193 / 235 / -10292	-3077 / -2486	1136 / -285 / -2406 / -2237
358	299 / -571	-14 / -1613	-88 / -1115	-1443 / -894	1115 / 233	-3609 / -500	-701 / -149 / -2 / -9922 / -10964
359	-1959 / 106 / -1378	199 / 399 / -701	1375 / -381 / -1115	1343 / 43 / -894	947 / 233 / -11527	884 / -500 / -10485	-1231 / -149 / -1
360	812 / 108 / -1378	-3596 / 399 / -701	-4399 / -381 / -1115	-744 / 43 / -894	-177 / 233 / -11154	-4082 / -500 / -10112	-422 / -149 / -2
361	-2345 / 108 / -1378	-3672 / 399 / -701	-4049 / -381 / -1115	-2073 / 43 / -894	-284 / 233 / -11527	-3835 / -500 / -10485	2063 / -149 / -1
362	-3293 / 108 / -1378	57 / 399 / -701	1133 / -381 / -1115	-4573 / 43 / -894	-5208 / 233 / -11527	-2706 / -500 / -10485	-83 / -149 / -1
363	-3353 / 106 / -1378	-4471 / 399 / -701	-2714 / -381 / -1115	-4619 / 43 / -894	-5249 / 233 / -11527	-2751 / -500 / -10485	-2922 / -149 / -1
364	-3596 / 108 / -1378	-7189 / 399 / -701	3911 / -381 / -1115	-7594 / 43 / -894	-7397 / 233 / -11527	-5749 / -500 / -10485	-6591 / -149 / -1
365	-4594 / 106 / -1378	-5697 / 399 / -701	-175 / -381 / -1115	-5783 / 43 / -894	-6389 / 233 / -11527	-3732 / -500 / -10485	-1388 / -149 / -1
366	-2266 / 108 / -1378	1163 / 399 / -701	-4376 / -381 / -1115	1540 / 43 / -894	2819 / 233 / -11527	728 / -500 / -10485	-1199 / -149 / -1

Table 4, Sheet 28/32

(Numerical data table — column identifiers 518, 519, 520, 521, 522, 523, 524, 526, 527, 528, 530, 531, 532, 533, 534; row identifiers 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381.)

Table 4, Sheet 27/32

	535	536	537	538	539	540	541	542	568	570	571	572	573	574	575
	-815 -249	561 -249	-177 -249	-3531 -249	-3532 -249	-2808 -249	-2662 -249	-1539 -243	-3620 -250	1075 -249	3302 -249	-2324 -249	-2088 -249	-2381 -249	-3297 -249
	377 -294	3597 -294	353 -294	-4213 -294	-4214 -294	-3488 -294	-3420 -294	-2198 -289	-3808 -295	-2674 -294	2248 -294	-2971 -294	2205 -294	2000 -294	-3888 -294
	-2291 -369	-3301 -369	-1720 -369	-611 -369	-1484 -369	-2925 -369	-2848 -369	-1600 -375	-2263 -370	396 -369	-2449 -369	-2443 -369	1849 -369	-2478 -369	-3385 -369
	1630 117	-2599 117	-2560 117	229 117	-2497 117	-55 117	-1645 117	1175 119	-1074 117	-1017 117	-3036 117	-1317 117	-1321 117	-1330 117	-2288 117
	-3281 359	755 359	-911 359	84 359	529 359	1233 359	-1496 359	-492 384	1540 359	-974 359	-3353 359	-1268 359	-1382 359	-1272 359	821 359
	-3539 96	-2512 96	-59 96	-1142 96	1503 96	474 96	-1539 96	-320 90	-3042 95	373 96	-3598 96	2615 96	809 96	1114 96	1116 96
	17 45	-531 45	-1802 45	34 45	1007 45	-1008 45	1497 45	202 51	-2759 45	-277 45	-3165 45	1215 45	995 45	1191 45	504 45
	-4307 394	-3791 394	902 394	-1558 394	1382 394	1084 394	-2559 394	560 393	-2284 393	-2156 394	-4225 394	-2432 394	-2521 394	844 394	-3421 394
	762 275	-246 275	167 275	1729 275	312 275	849 275	673 275	-219 279	-2079 277	-712 275	186 275	-982 275	-1174 275	-1002 275	-1971 275
	1430 -720	-2881 -720	725 -720	-3118 -720	-3120 -720	-2393 -720	-2388 -720	-1100 -715	-2716 -721	1101 -720	-2078 -720	975 -720	-1500 -720	-1947 -720	321 -720
	223 -466	-3711 -466	-1746 -466	-1661 -466	-4047 -466	-1031 -466	-68 -466	-1999 -471	-3599 -466	-2473 -466	986 -466	-2815 -466	-2303 -466	-2872 -466	672 -466
	-3421 210 *	-705 210 *	785 210 *	739 210 *	987 210 *	-107 210 *	-962 210 *	1436 205 *	-3117 212 *	1705 210 *	-3727 210 *	723 210 *	869 210 *	1621 210 *	1798 210 *
	-2390 -626 *	-1076 -626 *	-4124 -626 *	-1612 -626 *	201 -626 *	-3373 -626 *	-3308 -626 *	-2015 -632 *	-3308 -627 *	-2487 -626 *	-2480 -628 *	-2875 -626 *	640 -626 *	659 -626 *	-3812 -626 *
	232 106 -1378	-2380 106 -1378	-2259 106 -1378	-117 106 -451	-2190 106 -451	-1467 106 -64	-1205 106 -41	-243 100 -74	-2789 105 -248	-726 106 -42	-1573 106 -134	-968 106 -45	-1087 106 -145	-1023 106 -1694	-71 106 -1540
	-630 399 -701	2389 399 -701	1244 399 -701	-1340 399 -1898	698 399 -1898	-614 399 -1898	547 399 -5157	-2289 407 -4314	-1512 398 -2662	-2064 399 -5129	-4239 399 -3497	-2343 399 -5040	-2440 399 -3388	-2365 399 -533	-3328 399 -608
	1894 -381 -1115	-253 -381 -1115	-92 -381 -1115	334 -381 -1115	-4352 -381 -1115	-3623 -381 -1115	-3524 -381 -1115	972 40 -386	-3572 -381 -5048	2759 -381 -1115	1528 -381 -1115	-3146 -381 -1115	-2538 -381 -1115	-3176 -381 -1115	-570 -381 -1115
	20 43 -894	-2134 43 -894	-54 43 -894	-1002 43 -894	-52 43 -894	-1134 43 -894	911 43 -894	-3521	-3163 45 -44	1046 43 -894	-4013 43 -894	-685 43 -894	1183 43 -894	727 43 -894	-1664 43 -894
	-4241 233 -11527	-986 233 -11527	1550 233 -4386	2080 233 -11457	-704 233 -1133	1598 233 -847	2685 233 -1126	-432 234 -2354	-3035 232 -8518	292 233 -8503	1818 233 -9503	841 233 -9758	-1493 233 -9758	465 233 -9978	288 233 -11204
	2114 -500 -10485	-3754 -500 -10485	-4070 -500 -10485	-4029 -500 -10415	-4031 -500 -10415	-3304 -500 -9537	-3241 -500 -8368	-1975 -505 -632	-1244 -500 -8461	-2471 -500 -8461	-2830 -500 -8461	-2812 -500 -8715	-2365 -500 -8715	-2857 -500 -8936	-3773 -500 -10162
	268 -149 -1	-86 -149 -1	237 -149 -72	-63 -149 -2	-773 -149 -881	741 -149 -1176	-1675 -149 -893	1055 -151 -2651	3077 -147 -2640	837 -149 -6	-3113 -149 -6	1024 -149 -5	-1389 -149 -5	-252 -149 -4	835 -149 -2

Row labels: 382 .. 383 .. 384 .. 385 .. 386 .. 387 .. 388 .. 389 .. 390 .. 391 .. 392 .. 393 .. 394 .. 395 .. 396 ..

	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590
	1959 -249	-3503 -249	-2908 -249	-681 -249	2319 -249	-2900 -249	1656 -249	-503 -249	-2822 -249	-2918 -249	578 -249	-3592 -249	2967 -249	-6754 -249	877 -249
	-3543 -294	-4184 -294	-3287 -294	-3175 -294	1884 -294	-3267 -294	890 -294	870 -294	-3164 -294	-3266 -294	2168 -294	-4274 -294	-2264 -294	-6951 -294	647 -294
	986 -369	-3619 -369	-2284 -369	-566 -369	-314 -369	1282 -369	505 -369	-558 -369	-1289 -369	-2226 -369	-857 -369	-1727 -369	-1344 -369	-1415 -369	1067 -369
	-116 117	-495 117	-758 117	-2818 117	-794 117	257 117	-2851 117	653 117	-2821 117	2532 117	-2817 117	-287 117	-1418 117	-4041 117	2168 117
	-750 359	-1394 359	-795 359	-3496 359	-6 359	-142 359	-1749 359	-762 359	-3512 359	860 359	1072 359	-663 359	116 359	-1285 359	19 359
	-177 96	1325 96	-3454 96	-3952 96	-171 96	-3827 96	-4014 96	-3972 96	-3983 96	-1461 96	1338 96	1482 96	-1750 96	-437 96	-1081 96
	-2353 45	-526 45	1410 45	-750 45	-862 45	-3320 45	-3835 45	1015 45	-259 45	-3694 45	-3794 45	-56 45	356 45	-5548 45	-712 45
	-3925 394	-29 394	-4253 394	-4466 394	-4454 394	-4343 394	-4507 394	4472 394	-4477 394	-4463 394	-4472 394	2750 394	-2792 394	-5195 394	4447 394
	-665 275	-2145 275	66 275	-4034 275	1587 275	-1288 275	-4102 275	-4060 275	-1301 275	-3967 275	-1329 275	-524 275	540 275	-4794 275	966 275
	-193 -720	797 -720	2476 -720	-1920 -720	924 -720	571 -720	-1929 -720	2607 -720	1741 -720	573 -720	1453 -720	-3180 -720	-1061 -720	-5871 -720	475 -720
	530 -466	-1778 -466	2276 -466	585 -466	417 -466	-185 -466	1354 -466	1293 -466	2348 -466	817 -466	978 -466	-1839 -466	-235 -466	-6793 -466	-2578 -466
	-838 210	2491 210	-3309 210	-4122 210	-4070 210	-1305 210	-4215 210	-4164 210	-4181 210	-4015 210	-4168 210	-929 210	-1415 210	-8019 210	-4034 210
	1277 -626	-7 -626	-1220 -626	-415 -626	-1143 -626	886 -626	833 -626	2104 -626	-67 -626	-1241 -626	-705 -626	-1792 -626	-1493 -626	-6578 -626	1500 -626
	-2575 106	-2167 106	-1380 106	-3284 106	-3268 106	-3108 106	-3317 106	-3292 106	1872 106	-3299 106	2392 106	-2252 106	-1460 106	-5632 106	-346 106
	-353	-2206 399	-699 399	1064 399	-922 399	-765 399	-4458 399	-4422 399	-4427 399	272 399	-1169 399	-3593 399	1324 399	3826 399	-4395 399
	-1115	-1115	-1115	-369	-701	-701	-701	-701	-701	-701	-701	-701	-29	-701	-701
	-3221 -381	-4317 -381	-2836 -381	-2673 -381	-260 -381	-2798 -381	2973 -381	-2661 -381	1419 -381	-2774 -381	278 -381	-4411 -381	277 -381	-8763 -381	-975 -381
	-1115	-1115	-1115	-1115	-1115	-1115	-1115	-1115	-1115	-1115	-1115	-1115	-1115	-1115	-1115
	-864 43	-525 43	-3531 43	-4507 43	-4442 43	-350 43	-4620 43	-688 43	-4585 43	-4359 43	-4569 43	483 43	-1557 43	-5491 43	4396 43
	-894	-894	-894	-894	-894	-894	-894	-894	-894	-894	-894	-894	-894	-894	-894
	-879 233	1738 233	-4113 233	-1809 233	-5068 233	-4410 233	-5254 233	-5197 233	-5221 233	-4966 233	-5204 233	834 233	-2115 233	-1531 233	-5019 233
	-11428	-11426	-11489	-11527	-11527	-11527	-11527	-11527	-11527	-11527	-11527	-11527	-590	-9952	-11527
	-3170 -500	-3999 -500	-2854 -500	75 -500	-2722 -500	-2826 -500	-2736 -500	-2707 -500	-249 -500	-2789 -500	504 -500	-4091 -500	-1882 -500	-4239 -500	-2728 -500
	-10384	-10384	-10447	-10485	-10485	-10485	-10485	-10485	-10485	-10485	-10485	-10485	-10485	-8910	-10485
	823 -149	-2534 -149	-258 -149	2748 -149	1007 -149	2091 -149	-316 -149	-2877 -149	-102 -149	1121 -149	-1162 -149	-197 -149	108 -149	-1283 -149	-811 -149
	-2	-2	-2	-1	-1	-1	-1	-1	-1	-1	-1	-1	-4	-1	-1
	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411

Table 4, Sheet 28/32

Column headers **591–605** (second value is a column-constant; columns 604 and 605 carry a third constant value):

Row	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605
const	-249	-294	-369	117	359	96	45	394	275	-720	-466	210	-626	106 / -1378	399 / -701
412	-4852	-5170	-587	-483	-885	-5002	-4708	3885	-4554	-3960	-1705	-1470	-4375	-4650	-1375
413	1877	-3440	1009	-1390	-46	174	794	-1071	-493	-2214	159	-467	504	-2866	-4097
414	-5951	-6428	2105	232	-6511	-7081	-8860	-8917	-8819	288	-3594	-6962	3284	-7004	-7166
415	4339	-2818	-8169	-8976	-8634	-8768	-538	-7240	-5995	1998	-5320	-7401	-6013	-309	-7386
416	3906	1026	-4336	-3584	1462	-3807	1972	-4701	-46	-4070	-4822	-3410	-4733	-3510	-1214
417	-6442	-6941	-5576	-4212	525	-53	-4749	-38	-4169	-5927	-6784	-5288	-6677	-4973	3298
418	1627	-4300	-3737	1087	-2525	-747	1591	-65	-2247	-3206	-4131	-1860	-4185	1940	-3611
419	-936	-4231	-1215	-1030	-1370	-265	-1821	-3702	-542	1023	-1825	-681	-4056	-2271	-1292
420	1810	2033	-331	-2813	-491	-3927	2001	-4456	-844	905	-417	-4078	1638	58	-1417
421	1570	-4504	-3889	404	49	-2699	-2104	-1378	-2448	-3405	-1667	-2183	-4291	664	2854
422	382	2604	-1236	-830	-1247	445	413	-4284	-1012	3600	-4	-3495	-1113	-3056	-114
423	-3591	-4272	-1728	2247	744	-2341	-579	111	301	-225	-2200	-238	-1155	177	-387
424	638	-4177	-3615	-424	-2402	-2243	-162	-3587	1848	-3083	-4009	-51	-4063	-131	2430
425	393	-4188	500	-473	-580	-2233	613	-586	48	-3073	-4000	328	-4054	-153	1631
426	258	1977	-3583	-314	-106	533	-558	475	1373	113	-3977	1214	-4033	-168 / -282	489 / -2590

Five additional (unlabeled) leftmost columns, each carrying two constants:

Row	(−149 / −1)	(−500 / −10485)	(233 / −11527)	(43 / −894)	(−381 / −1115)
412	-463	-3750	-5545	-5211	-4778
413	-406	2352	-733	-3127	323
414	-4702	-4203	-7370	-7027	262
415	-7127	-6045	-7502	-7856	2309
416	-1722	-4302	-3604	-3359	-4183
417	-1586	-4606	633	-4189	-6820
418	-2645	-4116	2070	1526	-1241
419	-2625	-4031	-1060	3238	-618
420	-1034	-2720	-5079	-1279	1571
421	-2907	-4290	618	-962	-4563
422	-474	-1486	-4914	-1922	308
423	238	-4087	1128	-891	-4408
424	-1091	-3993	-3606	-817	75
425	-376	-3984	1222	-100	601
426	127	-3961	870	-877	-4283

Row labels at foot of table: 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426

Table 4, Sheet 29/32

Table 4, Sheet 30/32

Table 4, Sheet 31/32

Col	636	637	638	639	640	641	642	643	644	659	660	661	662	663	664
1	-3152 / -249	1195 / -249	1654 / -249	-3442 / -249	227 / -249	2123 / -249	670 / -249	-640 / -249	-2951 / -244	-1787 / -249	-2595 / -249	402 / -249	2663 / -249	-3359 / -249	853 / -249
2	-3759 / -294	2049 / -294	4459 / -294	-4124 / -294	1707 / -294	-3725 / -294	-4147 / -294	-4048 / -294	-3840 / -261	-2141 / -294	-3255 / -294	2785 / -294	-3560 / -294	-4041 / -294	-4154 / -294
3	-3065 / -369	-1510 / -369	-515 / -369	-723 / -369	-1049 / -369	-1416 / -369	-804 / -369	-3486 / -369	-3079 / -378	-78 / -369	-2651 / -369	-1752 / -369	-2769 / -369	-383 / -369	-527 / -369
4	385 / 117	-1039 / 117	33 / 117	-503 / 117	-2401 / 117	832 / 117	-812 / 117	982 / 117	739 / 125	1793 / 117	700 / 117	-885 / 117	-2280 / 117	205 / 117	-962 / 117
5	196 / 358	-1188 / 359	-3268 / 359	1074 / 359	-369 / 359	-520 / 359	-1403 / 359	-483 / 359	602 / 360	1106 / 359	-444 / 359	-1428 / 359	-451 / 359	175 / 359	-2393 / 359
6	-577 / 96	871 / 98	-3734 / 96	716 / 96	-488 / 96	-2447 / 96	609 / 96	1382 / 96	698 / 94	-488 / 96	477 / 98	-3408 / 96	1402 / 94	178 / 98	170 / 98
7	-1624 / 45	683 / 45	-908 / 45	599 / 45	-1774 / 45	-585 / 45	1063 / 45	1106 / 45	-1143 / 42	-2483 / 45	-871 / 45	-710 / 45	140 / 45	2036 / 45	1711 / 45
8	-3452 / 394	1881 / 394	-823 / 394	-1468 / 394	275 / 394	-3681 / 394	744 / 394	-3460 / 394	-3027 / 385	-3340 / 394	-26 / 394	-4001 / 394	-1131 / 394	37 / 394	-831 / 394
9	2087 / 275	908 / 275	-3820 / 275	1286 / 275	-2203 / 275	-506 / 275	1233 / 275	-738 / 275	1528 / 274	-2784 / 275	994 / 275	-3463 / 275	-2198 / 275	227 / 275	-137 / 275
10	2480 / -720	1222 / -720	1178 / -720	-3030 / -720	-2706 / -720	855 / -720	-3053 / -720	-235 / -720	-2550 / -701	910 / -720	512 / -720	224 / -720	-2394 / -720	4 / -720	-3057 / -720
11	-434 / -466	-3762 / -466	18 / -466	-1627 / -466	-3541 / -466	-505 / -466	-3980 / -466	-292 / -466	-3473 / -473	120 / -466	-648 / -466	1807 / -466	-1777 / -466	-244 / -466	-358 / -466
12	-1675 / 210	-1532 / 210	-3923 / 210	967 / 210	-1827 / 210	-113 / 210	896 / 210	913 / 210	-283 / 214	715 / 210	-914 / 210	41 / 210	824 / 210	612 / 210	105 / 210
13	-749 / -626	-1353 / -626	-952 / -626	-1114 / -626	-1653 / -626	749 / -626	-4035 / -626	-1451 / -626	-3530 / -633	-73 / -626	-3058 / -626	183 / -626	590 / -626	-277 / -626	-542 / -626

Row							
442	-848 / -149 / -2	-3504 / -500 / -10119	1165 / 233 / -11162	589 / -381 / -1115	-27 / 43 / -894	-2036 / 106 / -131	-713 / 399 / -3527
443	189 / -149 / -2	-3751 / -500 / -10119	-781 / 233 / -11162	-4059 / -381 / -1115	227 / 43 / -894	-1947 / 106 / -527	-151 / 399 / -1708
444	-54 / -149 / -2	-2472 / -500 / -10119	-1427 / 233 / -11264	1165 / -381 / -1115	-4321 / 43 / -894	-3054 / 106 / -671	248 / 399 / -1427
445	-818 / -149 / -2	-3941 / -500 / -10222	624 / 233 / -11264	779 / -381 / -1115	13 / 43 / -894	-146 / 106 / -235	-979 / 399 / -2734
446	812 / -149 / -50	2734 / -500 / -10311	1602 / 233 / -4899	-1033 / -381 / -1115	985 / 43 / -894	424 / 106 / -350	170 / 399 / -2217
447	-1344 / -149 / -2	-3417 / -500 / -10262	267 / 233 / -11304	889 / -381 / -1115	1427 / 43 / -894	-2287 / 106 / -551	-3595 / 399 / -1655
448	497 / -149 / -117	-3964 / -500 / -10289	-282 / 233 / -11331	28 / -381 / -1115	-89 / 43 / -894	2394 / 106 / -266	-3465 / 399 / -2571
449	446 / -149 / -618	-3864 / -500 / -10337	535 / 233 / -3698	-4185 / -381 / -1115	716 / 43 / -894	-2025 / 106 / -170	-1034 / 399 / -3170
450	-1976 / -147 / -1274	-3458 / -508 / -10222	2309 / 242 / -1525	-3776 / -387 / -188	892 / 49 / -3030	-1599 / 105 / -68	-61 / 396 / -4446
451	-159 / -149 / -4	-1691 / -500 / -1480	-3708 / 233 / -2132	1577 / -381 / -1115	-3099 / 43 / -894	-2133 / 108 / -111	-3286 / 399 / -3750
452	246 / -149 / -3	1159 / -500 / -9234	372 / 233 / -10276	318 / -381 / -1115	1702 / 43 / -894	-1318 / 108 / -1019	-2656 / 399 / -981
453	1069 / -149 / -2	-2335 / -500 / -9312	-4412 / 233 / -10354	28 / -381 / -1115	1 / 43 / -894	-2797 / 106 / -347	-3947 / 399 / -2224
454	277 / -149 / -210	-3251 / -500 / -10014	-2499 / 233 / -11056	1305 / -381 / -1115	-1946 / 43 / -894	1022 / 106 / -1716	-1730 / 399 / -524
455	-950 / -149 / -2	-3858 / -500 / -10101	39 / 233 / -2891	-4178 / -381 / -1115	480 / 43 / -894	-267 / 106 / -1071	-1336 / 399 / -932
456	728 / -149 / -2	-3966 / -500 / -10214	-624 / 233 / -11256	812 / -381 / -1115	909 / 43 / -894	597 / 108 / -297	-1165 / 399 / -2427

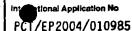|																					
457	66 -149 -2	-2581 -500 -10357	-5105 233 -11399	-4470 43 -894	-188 -381 -1115	-1573 399 -2427	-3183 106 -297	2992 -626	-1564 210	377 -466	-1794 -720	-3956 275	-4361 394	-3689 45	-3867 96	-107 359	-2705 117	805 -369	-3049 -294	493 -249	665
458	771 -149	-3980 -500	870 233	-586 43	-4301 -381	-3482 399	-2141 106 -297	-250 -626	2057 210	-2375 -466	-115 -720	-992 275	-830 394	1327 45	-288 96	497 359	102 117	-3602 -369	-4164 -294	-721 -249	666
459	824 -149 -2	-3981 -500 -10357	-2356 233 -11399	-848 43 -894	-4302 -381 -1115	961 399 -2427	-2141 106 -3104	-4052 -626	1359 210	-3997 -466	-3070 -720	753 275	459 394	-578 45	667 96	926 359	246 117	-3603 -369	956 -294	-564 -249	667
460	-262 -149 -2	504 -500 -10357	-5193 233 -11399	-4559 43 -894	-2662 -381 -1115	-1787 399 -701	-3291 106 -1378	-193 -626	-4160 210	1812 -466	2808 -720	-4058 275	-4471 394	-44 45	1834 96	-1315 359	-2817 117	405 -369	-3165 -294	-2822 -249	668
461	608 -149 -1	-3114 -500 -10485	1889 233 -11527	-2942 43 -894	-3135 -381 -1115	-1460 399 -701	-2791 106 -1378	1522 -626	-2790 210	-416 -466	-2294 -720	1311 275	-4111 394	-449 45	-3103 96	-610 359	-373 117	1203 -369	-3513 -294	-3084 -249	669
462	1064 -149 -1	-4092 -500 -10485	352 233 -11527	-287 43 -894	-4413 -381 -1115	692 399 -701	1287 106 -1378	-4164 -626	-609 210	-518 -466	-3181 -720	810 275	565 394	-79 45	69 96	879 359	-1440 117	-3714 -369	-4275 -294	333 -249	670
463	1044 -149 -1	-2707 -500 -10485	-5195 233 -11527	-1784 43 -894	2589 -381 -1115	-2059 399 -701	-3291 108 -1378	959 -626	-1193 210	1067 -466	-1910 -720	-4058 275	-4471 394	-3790 45	-3971 96	-646 359	-403 117	212 -369	1583 -294	-2821 -249	671
464	-5309 -5869 -6219	-1531	-6756	-5764	2106	-1505	-2098	-5643	8	-4432	-5670	-483	3918	-5184	-4930	-5825	-5467	-5419			672

0

Table 4, Sheet 32/32

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page **54** , line **32** .

B. IDENTIFICATION OF DEPOSIT	Further deposits are identified on an additional sheet ☐

Name of depositary institution
DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

Address of depositary institution *(including postal code and country)*
DSMZ
Mascheroder Weg 1b
D-38124 Braunschweig
Germany

Date of deposit	Accession Number
15/09/2003	DSM 15926

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)*	This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

⎯ For receiving Office use only ⎯	⎯ For International Bureau use only ⎯
☒ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer M. Szamock:	Authorized officer

Form PCT/RO/134 (July1998; reprint January 2004)

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   C12N5/10   A01H5/00   C12N15/82   C12N9/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7   C12N   A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, Sequence Search, BIOSIS, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/70942 A (DANISCO ; POULSON PETER (DK); SOERENSEN IBEN SCHILDT (DK)) 27 September 2001 (2001-09-27) the whole document	1-17,22, 23,26-32
X	WO 96/34968 A (GIDLEY MICHAEL JOHN ; WESTCOTT ROGER JOHN (GB); COOKE DAVID (GB); DEBE) 7 November 1996 (1996-11-07)	1-17,22, 23,26-32
X	example 25 example 1 page 28 page 39, paragraph 3 claims 1-68	18,23-26

-/--

X	Further documents are listed in the continuation of box C.		X	Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 January 2005	18/01/2005

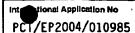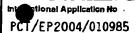| Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Holtorf, S

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! 8 June 2001 (2001-06-08), "EST512701 cSTD Solanum tuberosum cDNA clone cSTD203 5' sequence, mRNA sequence." XP002309391 retrieved from EBI accession no. EM_PRO:BG886850 Database accession no. BG886850 abstract	18-20,23
A	DATABASE UniProt 'Online! 1 March 2003 (2003-03-01), "Putative 1,4-alpha-glucan branching enzyme." XP002309392 retrieved from EBI accession no. UNIPROT:Q8GWK4 Database accession no. Q8GWK4 abstract & DATABASE EMBL SEQUENCE LIBRARY 13 December 2002 (2002-12-13), SEKI, M., ET AL.: "A. thaliana At3g20440 mRNA for putative 1,4-alpha-glucan branching enzyme, complete cds, clone: RAFL21-11-008" accession no. WWW.EBI.AC.UK Database accession no. AK118785 abstract	
A	JOBLING STEPHEN A ET AL: "A minor form of starch branching enzyme in potato (Solanum tuberosum L.) tubers has a major effect on starch structure: Cloning and characterisation of multiple forms of SBE A" PLANT JOURNAL, vol. 18, no. 2, April 1999 (1999-04), pages 163-171, XP002309385 ISSN: 0960-7412 figure 3 page 166, left-hand column, paragraph 1	
A	LARSSON CLAS-TOMAS ET AL: "Molecular cloning and characterization of starch-branching enzyme II from potato" PLANT MOLECULAR BIOLOGY, vol. 37, no. 3, June 1998 (1998-06), pages 505-511, XP002309386 ISSN: 0167-4412 the whole document figure 2	

-/--

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KHOSHNOODI JAMSHID ET AL: "The multiple forms of starch-branching enzyme I in Solanum tuberosum" EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 242, no. 1, 1996, pages 148-155, XP002309387 ISSN: 0014-2956 the whole document figure 1	
A	MIZUNO K ET AL: "ALTERATION OF THE STRUCTURAL PROPERTIES OF STARCH COMPONENTS BY THE LACK OF AN ISOFORM OF STARCH BRANCHING ENZYME IN RICE SEEDS" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 268, no. 25, 5 September 1993 (1993-09-05), pages 19084-19091, XP002014043 ISSN: 0021-9258 the whole document	
A	BLAUTH SUSAN L ET AL: "Identification of Mutator insertional mutants of starch-branching enzyme 1 (sbe1) in Zea mays L" PLANT MOLECULAR BIOLOGY, vol. 48, no. 3, February 2002 (2002-02), pages 287-297, XP002309388 ISSN: 0167-4412 page 295, right-hand column, last paragraph - page 296, left-hand column	
A	BLAUTH SUSAN L ET AL: "Identification of Mutator insertional mutants of starch-branching enzyme 2a in corn" PLANT PHYSIOLOGY (ROCKVILLE), vol. 125, no. 3, March 2001 (2001-03), pages 1396-1405, XP002309389 ISSN: 0032-0889 page 1401	
A	FLIPSE E ET AL: "INTRODUCTION OF SENSE AND ANTISENSE CDNA FOR BRANCHING ENZYME IN THE AMYLOSE-FREE POTATO MUTANT LEADS TO PHYSICO-CHEMICAL CHANGES IN THE STARCH" PLANTA, SPRINGER VERLAG, DE, vol. 198, 1996, pages 340-347, XP009019570 ISSN: 0032-0935 the whole document page 346, right-hand column, last paragraph	

-/--

1

Form PCT/ISA/210 (continuation of second sheet) (January 2004)

International Application No
PCT/EP2004/010985

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RYDBERG ULRIKA ET AL: "Comparison of starch branching enzyme I and II from potato" EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 268, no. 23, December 2001 (2001-12), pages 6140-6145, XP002309390 ISSN: 0014-2956 the whole document	

1

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0170942	A	27-09-2001	GB	2360521 A	26-09-2001
			AU	3950601 A	03-10-2001
			CA	2402463 A1	27-09-2001
			EP	1265477 A2	18-12-2002
			WO	0170942 A2	27-09-2001
			US	2004068766 A1	08-04-2004
WO 9634968	A	07-11-1996	AU	706009 B2	03-06-1999
			AU	5509996 A	21-11-1996
			CA	2217878 A1	07-11-1996
			CA	2416347 A1	07-11-1996
			EP	0826061 A2	04-03-1998
			WO	9634968 A2	07-11-1996
			US	2003166919 A1	04-09-2003
			US	6825342 B1	30-11-2004